VOCAL RECOGNITION AND SOCIAL COGNITION IN THE ACORN WOODPECKER

A Dissertation

Presented To The Faculty Of The Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Michael A. Pardo

August 2019

VOCAL RECOGNITION AND SOCIAL COGNITION IN THE ACORN WOODPECKER

Michael A. Pardo, Ph.D.

Cornell University 2019

The social intelligence hypothesis states that the social environment is the primary driver of the evolution of advanced cognition, and predicts that species with more complex social interactions will have more highly developed cognitive abilities. While this hypothesis is generally supported, the specific social selection pressures acting on cognitive evolution are less well understood. Acorn woodpeckers (*Melanerpes formicivorus*) are group-living birds in which alliances with kin and competition with other groups are often critical to fitness, suggesting that their social cognitive abilities may be particularly complex. In this dissertation, I investigated two pillars of social cognition in the acorn woodpecker: individual recognition and triadic awareness, or knowledge about social relationships between other individuals. In Chapter 1, I played back the calls of a current group member, a former group member, and a non-group member to male and female woodpeckers. While there were no clear differences in response among the three treatments, subjects responded more quickly to callers that had died or disappeared than to callers that were still living in the study area, suggesting that they recognize and monitor former group members post-dispersal. In Chapters 2 and 3, I investigated what acorn woodpeckers know about the relationships between members of other groups by playing back overlapping calls to simulate two individuals from outside the subject's group calling together, a behavior that normally only occurs between social affiliates. In Chapter 2, females responded more quickly to socially anomalous playbacks in which the callers belonged to two different groups compared to socially congruous playbacks in which the callers belonged to a single group, suggesting they recognize social affiliations between members of other groups. In

Chapter 3, females also discriminated between a pair of callers that formerly lived together and a pair of callers that never lived together, indicating that they can recognize relationships between individuals that have not lived together for years. These results suggest that social knowledge about members of other groups is particularly important for acorn woodpeckers, and highlight the importance of considering how social selection pressures external to the core social unit may have shaped the evolution of intelligence.

BIOGRAPHICAL SKETCH

Mickey Pardo's lifelong interest in wildlife began in early childhood with his first trip to the Los Angeles Zoo, and by elementary school he had decided that he would spend his life studying wild animals. He began conducting research on animal behavior as a homeschooled high school student, studying visual communication in Eastern gray squirrels in his parent's backyard. As an undergraduate student at the State University of New York College of Environmental Science and Forestry (SUNY-ESF), he continued this project under the guidance of Dr. Bill Shields, and ultimately developed it into an honors thesis and a peer-reviewed publication. In May 2012, Mickey graduated from SUNY-ESF *summa cum laude* with a B.S. in environmental biology, and in the summer following graduation, he worked as a field assistant studying toucan seed dispersal in Costa Rica.

In the fall of 2012, Mickey began his Ph.D. program in the Department of Neurobiology and Behavior at Cornell University, supervised by Dr. Walt Koenig. He began by studying vocal communication and social cognition in Asian elephants in Sri Lanka, but ultimately decided to switch to studying similar questions in the more tractable acorn woodpecker. Mickey has maintained a strong interest in elephants, and has published two papers on elephant behavior in addition to his work on acorn woodpeckers. After Walt's retirement, Dr. Mike Webster stepped in as Mickey's co-supervisor.

To the ikka birds, whose fascinating social lives won me over.

שתחיו חיים ארוכים ומשגשגים, נקרים יקרים.

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the help and support of so many individuals. I would like to thank my co-advisor, Walt Koenig, for allowing me the freedom to pursue own independent interests and for always providing rapid feedback and encouragement with a hearty “keep up the good work”. I would like to thank my other co-advisor, Mike Webster, for “adopting” me after Walt retired and welcoming me into WebLab. I would also like to thank my minor committee members, Janis Dickinson, Chris Clark, and Peter Wrege, for their advice and assistance throughout my Ph.D., and Eric Walters, who has been like a sixth committee member for me and provided invaluable feedback on most of my manuscripts. I owe a huge debt of gratitude to my incredible field assistants, Tejal Kuray, Emilee Sparks, Rob Anderson, and Sarah Heimbach. Without their hard work, I would not have been able to gather nearly as much data as I did, and their presence brightened my experience in the field. I am also grateful to Sahas Barve, Mario Pesendorfer, and Natasha Hagemeyer for sharing data, providing feedback on my manuscripts, and renaming hundreds of playback files so that my experiments would be properly blinded.

I thank Vince Voegeli, the former director of Hastings Reserve, for providing me with a speaker with which to conduct my playback experiments and for solving practically every maintenance problem under the sun, from the dishwasher that repeatedly flooded our kitchen to the swarm of bees that took up residence inside my house. I would also like to thank the staff members at the Lab of Ornithology, both past and present, who have gone above and beyond to help me with my research. It is impossible to name everyone, but I am especially grateful to Greg Budney, Russ Charif, Liz Rowland, Karl Fitzke, Bill McQuay, and Mike Pitzrick for teaching me how to record and analyze animal vocalizations and for providing invaluable

technical assistance. I thank the Macaulay Library for loaning me recording equipment every field season, and Lynn Johnson from the Cornell Statistical Consulting Unit for all of her help with data analysis. I also want to thank all the NB&B staff members who have guided me through the myriad administrative challenges of grad school, especially Terri Natoli, Saundra Anderson, and Sara Eddleman.

I am deeply grateful to all the friends I have made in both Ithaca and California, who helped keep me sane and supported me through some of the most difficult periods of the past seven years. And of course, I would not be where I am today were it not for my family. My dad has taught me most of what I know about statistics, and patiently helped me through countless data analysis challenges. My mom's support and advocacy on my behalf throughout my education has enabled me to succeed, and her help with formatting this dissertation was invaluable and greatly appreciated. Both my parents nurtured my early interest in wildlife and have always encouraged me to pursue my dreams. I would like to thank my brothers, Yudi and Jeremy, for all of their insight during our many conversations about biological research, and for being my best friends as well as my siblings.

I thank the following organizations for their financial support: the National Science Foundation (Graduate Research Fellowship and Doctoral Dissertation Improvement Grant IOS-1701451), the National Geographic Society (Young Explorer's Grant), the Animal Behavior Society, the American Society of Mammalogists, Sigma Xi, the International Society for Behavioral Ecology, the Department of Neurobiology and Behavior, the Cornell Graduate School, and the Cornell Lab of Ornithology (Athena Fund, Charles Walcott Graduate Fellowship, and Ivy Graduate Fellowship). I also thank the many individuals who generously contributed to a crowdfunding campaign that I launched in my second year.

Finally, I thank the acorn woodpeckers themselves, for the opportunity to watch the riveting dramas of their social lives, and for cooperating long enough for me to get the data I needed.

TABLE OF CONTENTS

Biographical Sketch……iii

Dedication……...iv

Acknowledgements……..v

Table of Contents……...vii

List of Figures……..x

List of Tables……..xi

Chapter 1: Responses to the calls of former group members in acorn woodpeckers

Abstract……1

Introduction……...1

Material and Methods……..5

(a) Study site and population monitoring……5

(b) Recordings of playback stimuli……5

(c) Playback protocol……..6

(d) Measuring response to playback……8

(e) Statistical analyses……8

Results……..9

(a) Response Latency……..9

(a) Approach Distance……...14

Discussion……..15

Responses to current, former, and non-group members….....15

Dead or Alive vs. Time Since Together…….15

Mechanism of recognition…….17

Conclusion…….18

Chapter 2: Wild acorn woodpeckers recognize associations between individuals in other groups

Abstract……..19

Introduction……19

Material and Methods……23

(a) Study site and population monitoring……...23

(b) Experimental design…….23

(c) Playback protocol…….26

(d) Recordings of playback stimuli…….....28

(e) Construction of playback stimuli……..29

(f) Measuring degree of synchrony within playback stimuli…….30

(g) Characterizing amplitude of playback system…….35

(h) Measuring response to playback……..36

(i) Predictions…….37

(j) Statistical analyses……38

Results……39

(a) Response of the focal breeder female……..42

(b) Responses of other birds……..43

(c) Circular mean of angular moment……...43

Discussion……..43

Chapter 3: Female acorn woodpeckers recognize relationships between third parties that have not lived together for years

Abstract……..48

Introduction……49

Material and Methods……54

(a) Study site and population monitoring……...54

(b) Experimental design…….55

(c) Selection of calls for playback stimuli…….57

(d) Construction of playback stimuli…….58

(e) Measuring response to playback……..60

(f) Statistical analyses……60

Results……60

(a) Latency to Directional Flight……60

(b) Latency to React……...62

(c) Distance of First Approach……..62

(d) Post-hoc analysis……..64

Discussion……..67

References……..73

LIST OF FIGURES

Figure 1.1: Latency to respond to live vs. dead/missing callers, Ch. 1……10

Figure 1.2: Latency to respond to current, former, and non-group members……...11

Figure 1.3: Latency to respond to live vs. dead former group members, and latency as a function of time since subject and caller last lived together……13

Figure 1.4: Latency to respond to same vs. opposite-sex non-group members……14

Figure 2.1: Spectrogram of playback stimulus, Ch. 2……..33

Figure 2.2: Latency to respond to socially incongruous vs. congruous playbacks……...42

Figure 3.1: Predictions for each hypothesis, Ch. 3……...53

Figure 3.2: *Latency to Directional Flight* in response to each treatment, Ch. 3……...61

Figure 3.3: *Latency to React* in response to each treatment, Ch. 3……...62

Figure 3.4: *Distance of First Approach* in response to each treatment, Ch. 3……..63

Figure 3.5: Latency to respond to live vs. dead/missing callers, Ch. 3……66

LIST OF TABLES

Table 1.1: Experimental design for Ch. 1……...7

Table 1.2: Model outputs for Ch. 1……...12

Table 2.1: Experimental design for Ch. 2…….25

Table 2.2: Definitions of response variables, Ch. 2……..37

Table 2.3: Outputs for reduced models, Ch. 2……..40

Table 2.4: Outputs for full models, Ch. 2…….41

Table 3.1: Model outputs for Ch. 3……...64

CHAPTER 1

RESPONSES TO THE CALLS OF FORMER GROUP MEMBERS IN ACORN WOODPECKERS

Abstract

In species with long-term social relationships, an ability to recognize individuals after extended separation can be evolutionarily beneficial. It could also be advantageous to distinguish between former social affiliates that have died and those that have left the group but may return, yet few studies have investigated whether animals can make this discrimination. I presented individual acorn woodpeckers, a cooperatively breeding, group-living bird, with three playbacks: current group member, former group member, and non-group member. Some subjects received the call of a former group member currently living in a nearby group, while others received the call of a former group member that was no longer present in the study area and thus presumed dead. Responses to current, former, and non-group members did not differ significantly. However, subjects responded significantly more quickly to presumed dead callers than to living callers. These results are consistent with the hypothesis that acorn woodpeckers continue to interact with former group members post-dispersal and/or remember former social affiliates for several years, and that they may be able to determine which former group members are still alive.

Introduction

In many long-lived animals, individuals repeatedly interact with the same conspecifics for protracted periods, and interactions may be separated by multiple years with no contact. An ability to remember individual conspecifics after extended periods of separation can thus be critical for maintaining long-term social relationships, as has been documented in a number of species. Male hooded warblers *(Wilsonia citrina)*, for example, recognize the songs of territorial

neighbors not seen for 8 months because of winter migration (Godard 1991). Some otariid pinnipeds (Insley 2000; Pitcher et al. 2010) and primates (Matthews and Snowdon 2011; Keenan et al. 2016) remember the calls of individual conspecifics for at least several years, and captive bottlenose dolphins (*Tursiops truncatus*) can recognize the whistles of former tank mates two decades after separation (Bruck 2013). Common ravens (*Corvus corax*) not only recognize individual conspecifics based on vocal cues for several years, but also remember the nature of the former relationship (Boeckle and Bugnyar 2012).

In the visual modality, domestic sheep *(Ovis aries)* can remember the faces of at least fifty conspecifics for over two years (Kendrick et al. 2001), and orangutans *(Pongo pygmaeus)* can recognize the faces of former social affiliates for at least ten years (Hanazuka et al. 2013). Other species, however, fail to show evidence of long-term social recognition in certain contexts. Yearling Belding's ground squirrels *(Urocitellus beldingi)* recognized the scent of former littermates after hibernation, which could be due to either long-term social memory or phenotype matching, but they failed to recognize the scent of previously familiar non-kin (Mateo and Johnston 2000).

Despite the benefits of long-term recognition, remembering other individuals indefinitely without updating knowledge about them is presumably costly. Long-term memory capacity can be limited (Cook et al. 2005), and there could be additional costs to retaining outdated information, as former alliance partners that have died or permanently left the area can no longer be relied upon for assistance. Thus, animals may be under selection to recognize when former social affiliates have died or to forget them entirely once a threshold of time has elapsed with no interaction. Primates, cetaceans, corvids, and elephants show particular interest in the corpses of conspecifics and respond differently to them than to living conspecifics or heterospecific

corpses, raising the possibility that they have an awareness of death (Gonçalves and Biro 2018). However, experimental evidence is lacking as to whether any species forms separate mental categories for dead and living former social affiliates.

Acorn woodpeckers (*Melanerpes formicivorus*) provide an excellent system in which to study social recognition. In California, this species lives in family groups on stable, year-round territories (MacRoberts and MacRoberts 1976). Groups consist of one to four breeding females and one to eight breeding males, all mating with one another and cooperatively raising the offspring in a single nest (Koenig et al. 2016). Cobreeders of the same sex are typically close relatives, but opposite sex breeders are unrelated (Koenig et al. 1998). Because suitable breeding locations are limited, offspring of both sexes may also remain in their natal territory for many years as nonbreeding helpers (Koenig et al. 2011). Opportunities for helpers to disperse and breed occur when all the breeders of a particular sex have died or disappeared from another group, creating a breeding vacancy. Helpers (and sometimes breeders) from multiple groups often engage in "power struggles" to claim such vacancies, and individuals form coalitions with same-sex kin to improve their chances of competing successfully (Koenig 1981a; Hannon et al. 1985).

Acorn woodpeckers can discriminate between vocalizations of their current group members and those of members of other groups (Yao 2008), and several observations suggest that former group-mates may still recognize one another several years after one of them has dispersed. First, individuals that have already attained a breeding position may temporarily leave their territory to help relatives with which they have not lived for a year or more to fight for a breeding vacancy elsewhere (N. Hagemeyer, pers. comm.). Second, individuals may re-join their natal group many years after dispersing (W. Koenig. and E. Walters, unpublished data). Third,

individuals may disperse into a group with established breeders if the breeders of the same sex as themselves are their former group-mates. If acorn woodpeckers recognize former group members, it could also be beneficial for them to distinguish between former group members that have died or dispersed far away, and former group members that have dispersed to nearby groups and can thus continue to interact with them, at least occasionally. For example, helpers seeking to join kin that have already attained breeder status in another group require up-to-date knowledge about which of their former group members are still alive and present in the vicinity. Similarly, it is possible that individuals sometimes actively recruit kin living in other territories to form coalitions, in which case they would need to know which individuals are still alive and able to offer assistance.

I investigated whether acorn woodpeckers can discriminate among the calls of current group members, former group members, and non-group members, and whether they can distinguish presumed dead from living former group members based on vocal cues. I predicted that both sexes would react more quickly and approach the speaker more closely in response to playbacks of non-group members compared to playbacks of current or former group members. I further predicted that if acorn woodpeckers can distinguish presumed dead and living former group members, they would react more quickly and approach more closely in response to callers that are presumed dead. I also predicted that if recognition of former group members attenuates with time, subjects should respond more quickly and approach more closely to former group members from which they had been living separately for longer.

As acorn woodpeckers compete with same-sex rivals for breeding vacancies (Hannon et al. 1985), I predicted that both males and females would react more quickly and approach more closely in response to non-group members of the same sex as themselves compared to non-group

members of the opposite sex. Moreover, given that females are more likely than males to engage in power struggles (Koenig 1981a), I predicted that females would react more quickly and approach more closely than males in response to same-sex non-group members.

Material and Methods

(a) *Study site and population monitoring*

I conducted a playback experiment with wild acorn woodpeckers at Hastings Natural History Reservation in central coastal California, USA (36.379ºN, 121.567ºW). This population has been the subject of a long-term study since 1968 (MacRoberts and MacRoberts 1976; Koenig 1981b), and >95% of the individuals are color-banded. Most individuals are banded in the nest at 21 days of age, and un-banded adults are captured to be banded whenever possible. There are approximately 50 social groups within the study area, and each group is censused every 8-10 weeks using spotting scopes to re-sight color bands.

Subjects for the current playback experiment were seven females and eight males from a total of nine different social groups. All subjects but one female were of breeder status. I conducted playback trials from 14 April to 19 June 2017, and from 2 May to 14 June 2018. All values given are mean ± standard deviation unless otherwise stated.

(b) *Recordings of playback stimuli*

I presented each subject with three different playback stimuli: the call of a current group member; the call of a former group member that had died, left the group, or remained on the natal territory after the subject had dispersed 1.1-6.4 years prior to the experiment (median = 2.5 years); and the call of an individual from a nearby territory that had never been a member of the subject's group. I used a total of 14 calls from 14 individuals as "current group member" stimuli, 14 calls from 14 individuals as "former group member" stimuli, and 13 calls from 12 individuals

as “non-group member” stimuli. The calls I used as playback stimuli were *waka* calls, an individually specific, affiliative call typically produced when members of the same group approach one another after a short period of separation (MacRoberts and MacRoberts 1976; Yao 2008). All playback stimuli were recorded at Hastings Reservation between 19 Mar 2015 and 26 May 2017 using a Sennheiser ME67 or ME62 microphone (Wedemark, Germany) and a Marantz PMD661 (Kanagawa, Japan), Fostex FR-2 (Akishima City, Tokyo, Japan), or Roland R26 (Hamamatsu, Shizuoka, Japan) digital recorder (48 kHz, 16 or 24 bits). Prior to constructing the playback stimuli, the calls were high-pass filtered (200 Hz cut-off, 6 dB roll off) and normalized to -3 dB in Audacity® 2.1.1, and any calls originally recorded at 24 bits were converted to 16 bits. The playback stimuli consisted of 60 sec of background noise with a 10 sec fade-in, followed by a single *waka* call, followed by 30 sec of background noise, followed by the same *waka* call, followed by a final 10 sec of background noise with a fade-out applied to all 10 sec.

(c) *Playback protocol*

Playback trials followed a similar protocol to Pardo et al. (2018). In brief, I placed a Yamaha PDX 11 loudspeaker (Hamamatsu, Shizuoka, Japan) calibrated to 100.1 ± 1.3 dB re 20 µPa at 1 m in a tree 1-1.5 m off the ground and 40 m away from a tree near the center of the group’s territory (“center tree”). Once the subject was located in the center tree, I began filming the subject using a Canon PowerShot SX510 digital camera (Ota City, Tokyo, Japan), and immediately played the appropriate playback file, with the 60 sec of background noise at the beginning of the file serving as a pre-playback period. In 2017, I also simultaneously dictated the subject’s behavior into a digital recorder.

Successive playbacks to the same group or to groups < 250 m from each other were spaced by 6.0 ± 4.8 days on average (minimum three days to same group, two days to groups

closer than 250 m) to reduce the risk of habituation. The three stimuli played to a given subject were always recorded from callers of the same sex. Four out of the seven female subjects and four out of the eight male subjects received playbacks of male calls, while the remaining three females and four males received playbacks of female calls. The order of presentation of the three stimuli was balanced as much as possible using an incomplete Latin square design that simultaneously controlled for *Order*, *Subject Sex*, and *Caller Sex* (Table 1.1).

Table 1.1. Experimental design to test if acorn woodpeckers could distinguish between the calls of current group members, former group members, and non-group members. *Order* refers to the order in which a given subject received the three playback stimuli (Current group member, Former group member, Non-group member). As much as possible, *Order* was balanced with respect to *Subject Sex*, *Caller Sex*, and whether the caller used for the "former group member" stimulus was dead or alive at the time of the experiment. *Dead or Alive* was not balanced with respect to *Subject Sex* or *Caller Sex.*

Subject ID	Group	Year	Order	Subject sex	Caller sex	Former group member dead or alive
F4672	1500	2017	C-F-N	F	F	Alive
F5007	KNOL	2017	C-F-N	F	F	Alive
F5945	PLQE	2018	C-F-N	F	M	Dead
M4754	MLF2	2017	C-F-N	M	M	Alive
M4935	CAVI	2018	C-F-N	M	F	Dead
F5389	ROBH	2017	F-N-C	F	M	Alive
F5103	PLQE	2017	F-N-C	F	M	Alive
M5006	PLQE	2017	F-N-C	M	M	Dead
M4889	LOLF	2017	F-N-C	M	F	N/A[1]
M3399	KNOL	2018	F-N-C	M	F	Dead
F5715	KNOL	2018	N-C-F	F	F	Dead
F4629	CAVI	2017	N-C-F	F	M	Alive
M5378	Y	2017	N-C-F	M	M	Alive
M5082	1500	2018	N-C-F	M	M	Alive
M5251	LHAY	2017	N-C-F	M	F	Dead

[1] For subject M4889 I mistakenly played the call of a "former group member" that had never actually lived with the subject, so this trial was excluded from analysis

Testing the difference in response to living callers vs. callers that had died or left the study area (hereafter "presumed dead") was not an *a priori* goal of this study. However, due to

the difficulty of obtaining playback-quality recordings from known individuals, I used the call of an individual that was presumed dead at the time of the experiment as the "former group member" stimulus for two female and four male subjects (Table 1.1). It is rarely possible to know for certain if an individual has died when studying a wild population, but none of the former group members that I classified as "dead" had been observed on the study site for at least a year prior to the experiment and none have been seen since (as of April 2019). For two of the males that received a "former group member" stimulus from a presumed dead caller, the "non-group member" stimulus was also from a presumed dead caller (not seen for seven months prior to the experiment).

(d) *Measuring response to playback*

Using the video and audio recordings of each playback trial, I measured the following aspects of the focal bird's response: latency to the first "directional" flight (defined as flying up to a higher vantage point or towards the speaker), latency to the first approach to the speaker, latency to the closest approach to the speaker, distance of the first approach to the speaker, and distance of the closest approach. For the latency variables, if the focal bird did not exhibit the behavior of interest within three min after the start of the playback, latency was assigned the maximum possible value of 180 sec and marked as censored. I was blind to the experimental condition in each trial until all scoring was complete.

(e) *Statistical analyses*

I conducted statistical analyses in R 3.5.1 (R Core Team 2018). The three latency response variables were all highly correlated (Pearson's $r>0.9$), so I only included *Latency to First Directional Flight* (hereafter referred to as *Response Latency*) in the analysis. The two distance variables were also highly correlated (Pearson's $r=0.94$), so I only included *Distance of*

First Approach (hereafter referred to as *Approach Distance*) in the analysis. I analyzed *Response Latency* using Cox regression in the R packages "*survival*" (Therneau 2015) and "*coxme*" (Therneau 2018) to account for the fact that some of the latencies were censored. I analyzed *Approach Distance* using linear mixed models using the R package "*lme4*" (Bates et al. 2015). I ran a model for each response variable (*Response Latency* and *Approach Distance*) with *Treatment* and *Dead or Alive* (whether the caller was alive or presumed dead) as fixed effects, and *Individual ID* as a random effect.

Among the "former group member" stimuli, *Dead or Alive* was confounded with the amount of time elapsed since the caller and the subject last lived in the same group (*Time Since Together*), because *Time Since Together* was longer for all but one of the living callers than for the callers that were presumed dead (*t*-test, $t_{8.8}$=3.4, *P*=0.008). To determine if *Dead or Alive* and *Time Since Together* had similar effects on *Response Latency*, I ran a separate model for both of these variables with *Response Latency* as the response.

Finally, to determine whether males and females differed in their response to territorial intruders from another group, I ran one model for each response variable (*Response Latency* and *Approach Distance*) using only the "non-group member" trials, with *Subject Sex*, *Same or Opposite Sex* (whether the subject and caller were of the same sex), and the interaction of these two variables as fixed effects. I then conducted pairwise comparisons for the interaction effect using Tukey's method in the R package "*emmeans*" (Lenth 2018).

Results

(a) *Response Latency*

Subjects responded significantly more quickly to the calls of presumed dead individuals than to the calls of living individuals (Cox regression, *N*=15, χ^2=7.1, *P*=0.008) (Figure 1.1).

However, there was no difference in *Response Latency* to current, former, or non-group members (Cox regression, N=15, χ^2=2.8, P=0.24) (Figure 1.2) (Table 1.2).

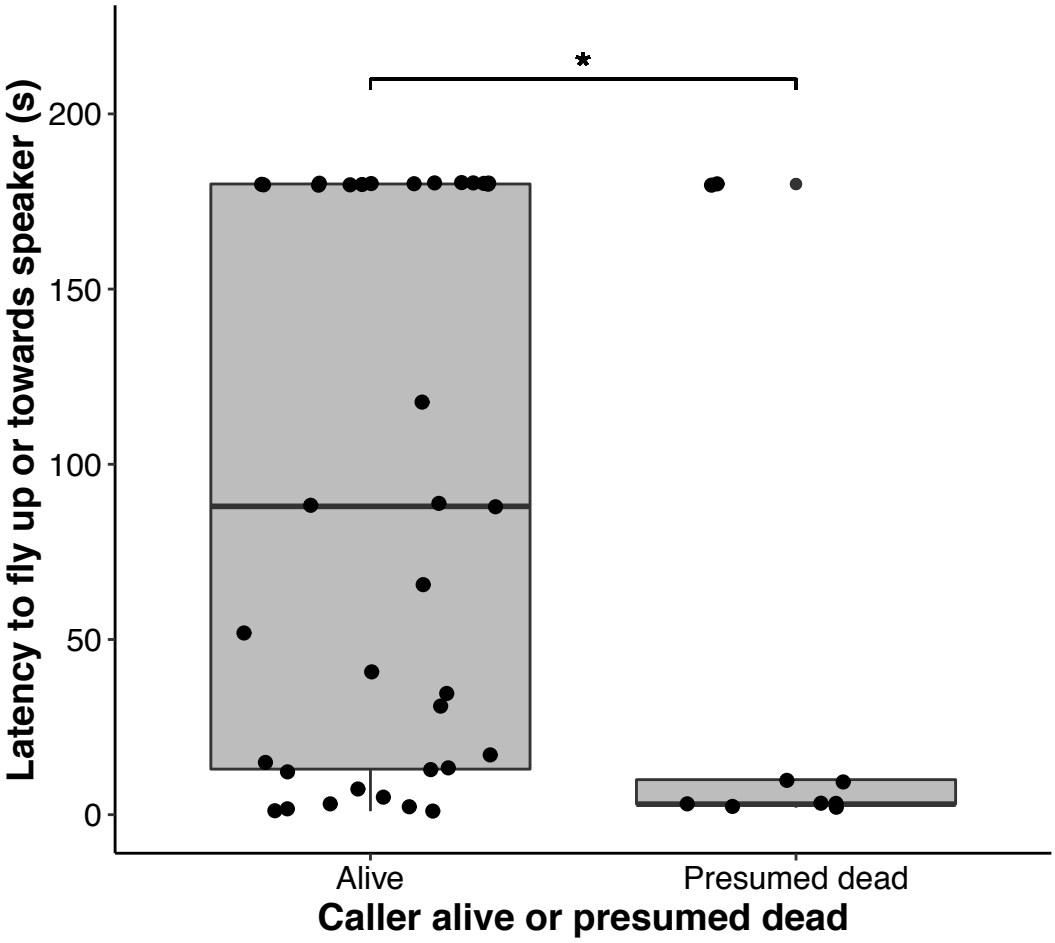


Figure 1.1. *Response Latency* as a function of whether the caller was alive or presumed dead at the time of the experiment. The data from all three treatment categories are included here, not just the "former group member" trials. * indicates P<0.05.

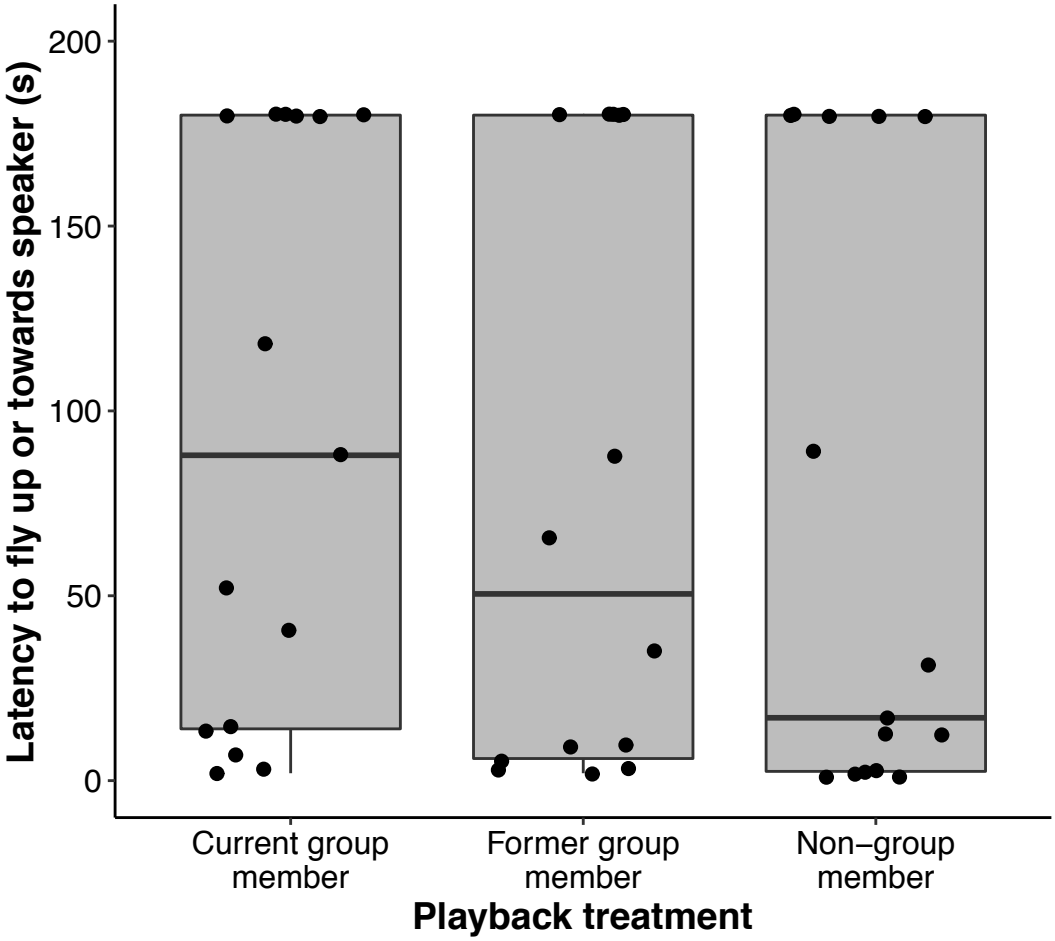


Figure 1.2. *Response Latency* as a function of *Treatment*. There were no significant differences in response among any of the three treatment categories.

Table 1.2. Output [Chi-square statistic (*P*-value) for fixed effects, variance for *Individual ID*] for each of the models.

Model Type	**Response**	**Treatment (df=2)**	**Dead/Alive (df=1)**	**Time Since Together (df=1)**	**Sex (df=1)**	**Same/Opp. Sex (df=1)**	**Sex* Same/Opp. Sex (df=1)**	**Individual ID**
Cox PH	Response Latency	2.8 (0.24)	7.1 (0.008)	--	--	--	--	0.46
Linear	Approach Distance	0.67 (0.72)	0.95 (0.33)	--	--	--	--	13.66
Cox PH	Response Latency ("former member" trials)	--	3.6 (0.06)	--	--	--	--	--
Cox PH	Response Latency ("former member" trials)	--	--	4.8 (0.03)	--	--	--	--
Cox PH	Response Latency ("non-member" trials)	--	--	--	0.01 (0.91)	5.1 (0.02)	2.0 (0.15)	--
Linear	Approach Distance ("non-member" trials)	--	--	--	0.60 (0.46)	0.37 (0.56)	0.17 (0.69)	--

Among the "former group member" trials alone, the relationship between *Response Latency* and *Dead or Alive* was not significant (Cox regression, N=14, χ^2=3.6, P=0.06) (Figure 1.3a). However, there was a significant relationship between *Response Latency* and *Time Since Together*, with subjects responding more quickly to former group members that had died or left the group more recently (Cox regression, N=14, χ^2=4.8, P=0.03) (Figure 1.3b).

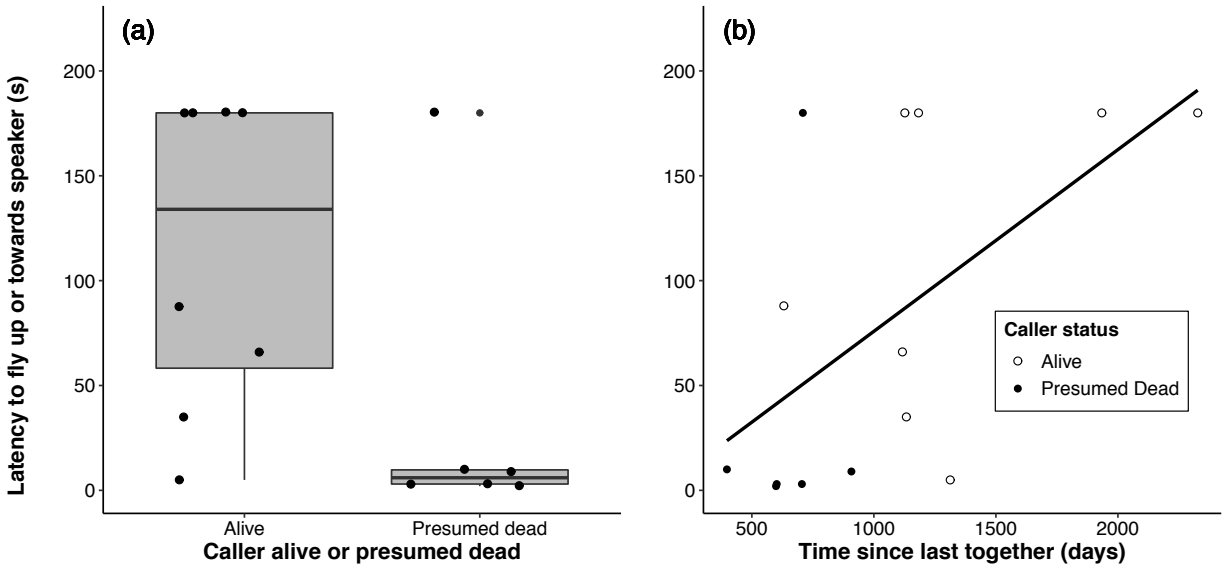


Figure 1.3. Among the "former group member" trials only: *Response Latency* as a function of (a) whether the caller was alive or presumed dead, and (b) the number of days since the subject and the caller last lived together. Open circles in (b) represent living former group members and filled circles represent presumed dead former group members. *Response Latency* was significantly related to *Time Since Together* (P=0.03) but not to *Dead or Alive* (P=0.06).

In "non-group member" trials, subjects responded more quickly to non-group members of their own sex than to opposite-sex non-group members (Cox regression, N=15, χ^2=5.1, P=0.02) (Figure 1.4). Males and females did not differ in their latency to respond to the playback (Cox regression, N=15, χ^2=0.01, P=0.91), and there was no interaction between *Subject Sex* and

whether the subject and caller were of the same sex (*Same or Opposite Sex*) (Cox regression, N=15, χ^2=2.0, P=0.15).

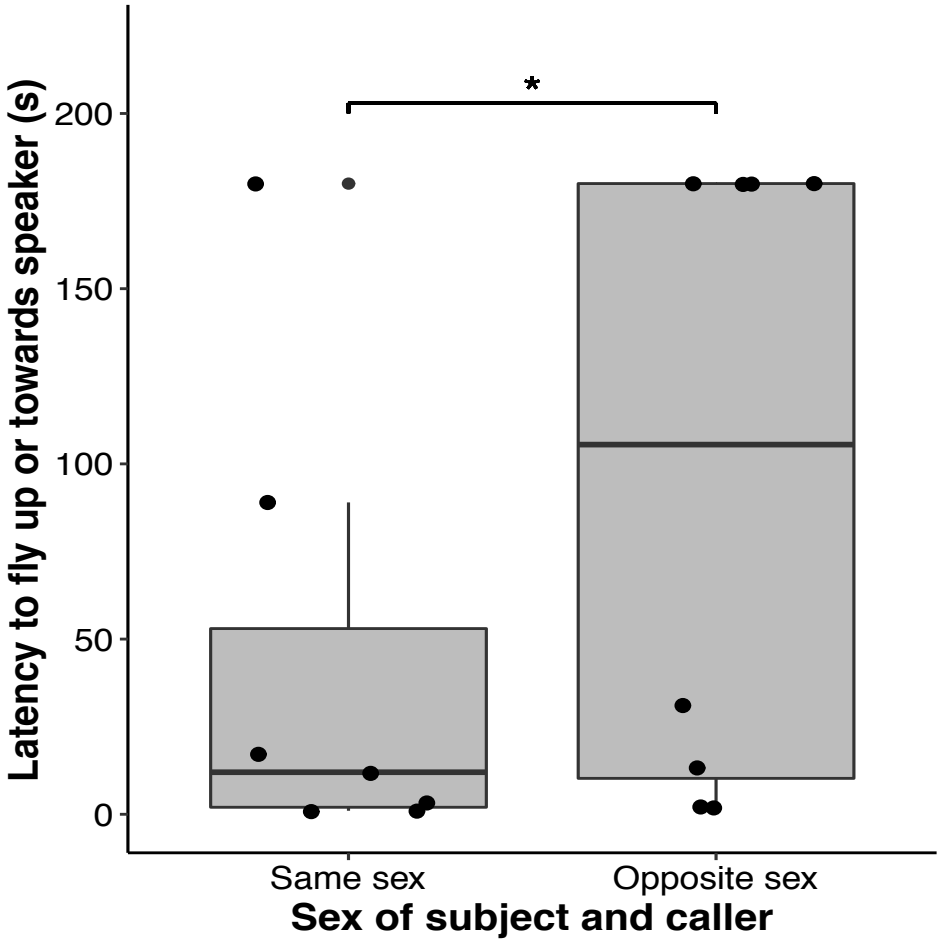


Figure 1.4. *Response Latency* to the calls of non-group members only, as a function of whether the subject and caller were of the same sex or opposite sex. All non-group member trials are included in this figure. * indicates P<0.05.

(b) *Approach Distance*

The distance of the subject's first approach to the speaker did not differ significantly among current, former, and non-group member playbacks (Cox regression, N=15, χ^2=0.67, P=0.72). *Approach Distance* also did not differ between playbacks of living callers and

playbacks of presumed dead callers (Cox regression, N=15, χ^2=0.95, P=0.33). Among the "non-group member" trials, *Approach Distance* was not significantly affected by *Subject Sex* (ANOVA, N=15, $F_{1,11}$=0.60, P=0.46), *Same or Opposite Sex* (ANOVA, N=15, $F_{1,11}$=0.37, P=0.56), or their interaction (ANOVA, N=15, $F_{1,11}$=0.17, P=0.69).

Discussion

Responses to current, former, and non-group members

Although subjects did not respond differently to current, former, and non-group members, the fact that subjects responded differently to the calls of individuals that were presumed dead and the calls of individuals that were still alive suggests that they vocally recognized at least some of their former group members. Moreover, in a previous study acorn woodpeckers responded more aggressively to the calls of non-group members than to the calls of current group members (Yao 2008). The discrepancy between this previous study and the current study could be due to methodological differences. While I broadcast playbacks from 40 m away from the subject, the previous study broadcast playbacks from a speaker in the same tree as the subject. Subjects might be more threatened by non-group member playbacks broadcast from a closer distance, and thus the difference in response to current and non-group members might be greater when the speaker is placed in the same tree as the subject.

Dead or Alive vs. Time Since Together

A particularly intriguing result of my study was the difference in response to the calls of presumed dead vs. living conspecifics. Few previous studies have investigated whether animals can distinguish between living and presumed dead former social affiliates. In one experiment, a single elephant family (*Loxodonta africana*) gave contact calls and approached the speaker in response to the call of a dead family member 23 months after her death, just as they would to a

living family member (McComb et al. 2000). This suggests that elephants may remember social affiliates for up to two years, but does not bear on whether or not they know that a social affiliate has died. Another study found that male chacma baboons (*Papio ursinus*) normally respond to the calls of close female associates, but fail to do so if the female's infant has recently died (Palombit et al. 1997). While this might indicate awareness of the infant's death, it could also be due to a change in the female's behavior following the death of her infant.

Because *Dead or Alive* and *Time Since Together* were confounded, it is not possible to definitively determine whether the acorn woodpeckers in the current study were able to distinguish between living and presumed dead former group members, or whether they were reacting to the fact that the presumed dead former group members were also ones they had last lived with more recently. However, the direction of the effect suggests that subjects were responding to *Dead or Alive*, not to *Time Since Together*. If subjects simply forgot former group members that had been absent from the group for longer, they should have treated them like non-group members by reacting more quickly. Instead, I observed the opposite; subjects reacted more quickly to former group members that had left the group more recently (which also happened to be the presumed dead former group members), and reacted more slowly to former group members that had left the group longer ago (which also happened to be the living former group members). These observations are consistent with the interpretation that subjects can distinguish between the calls of living and dead conspecifics. If acorn woodpeckers know which individuals are still living in the vicinity, they would be expected to react more quickly to the call of an individual that has been presumed dead for a year or more, either because they are surprised to hear the call of an individual they know to be dead, or because they no longer remember the dead individual and perceive it as a stranger (which merits an aggressive response).

Mechanism of recognition

There are at least two ways in which acorn woodpeckers could recognize former group members. One possibility is that they remember the calls of former group members for extended periods of time in the absence of any reinforcing interaction. Under this scenario, subjects may have responded more quickly to presumed dead former group members because their expectations were violated by hearing the call of a presumed dead individual that they recognized.

The mean time since a subject and "former group member" caller last lived together in this study was 2.9 years overall, and 3.7 years among the living former group members, with a maximum of 6.4 years. Some songbirds recognize their territorial neighbors after winter migration, which typically represents a separation of 8-9 months (Godard 1991; Draganoiu et al. 2014), and in one study, common ravens recognized former social affiliates after a mean of 2 years with no interaction (maximum = 3 years), although that study did not determine the upper limit of ravens' social memory (Boeckle and Bugnyar 2012). Thus, if the acorn woodpeckers in this study recognized their former group members via long-term memory, this would be among the longest examples of social memory yet demonstrated in a bird.

Alternatively, acorn woodpeckers could recognize former group members by continuing to interact with them after dispersal to another group. Radio-telemetry has revealed that acorn woodpeckers make numerous visits to the territories of other groups, giving them the opportunity to maintain contact with former group members (Koenig et al. 1996), and a number of other species, such as long-tailed tits (*Aegithalos caudatus*) and western bluebirds (*Sialia mexicana*) are known to interact with kin after dispersal (Hatchwell et al. 2001; Akçay et al. 2013). Under this scenario, acorn woodpeckers might forget former social affiliates after a certain amount of

time has passed with no interaction, and thus subjects may have reacted more quickly to presumed dead individuals because they mistook them for strangers and were responding aggressively. Even though more time had passed on average since the living former group members last lived in the same group as the subject compared to when the presumed dead former group members last lived with the subject, subjects had the opportunity to continue interacting with living former group members after dispersal, whereas this is obviously not the case for former group members that died.

Conclusion

This study suggests that acorn woodpeckers can vocally recognize at least some former group members, and may distinguish the calls of dead and living individuals. Future work should control for the amount of time since the subject and caller last lived in the same group. If acorn woodpeckers are found to discriminate between dead and living former group members independent of the time since they last lived in the same group, future studies should also examine the cognitive mechanism by which the woodpeckers make this discrimination.

CHAPTER 2

WILD ACORN WOODPECKERS RECOGNIZE ASSOCIATIONS BETWEEN INDIVIDUALS IN OTHER GROUPS

Abstract

According to the social intelligence hypothesis, understanding the cognitive demands of the social environment is key to understanding the evolution of intelligence. Many important socio-cognitive abilities, however, have primarily been studied in a narrow subset of the social environment—within-group social interactions—despite the fact that between-group social interactions often have a substantial effect on fitness. In particular, triadic awareness (knowledge about the relationships and associations between others) is critical for navigating many types of complex social interactions, yet no existing study has investigated whether wild animals can track associations between members of other social groups. I investigated inter-group triadic awareness in wild acorn woodpeckers (*Melanerpes formicivorus*), a socially complex group-living bird. I presented woodpeckers with socially incongruous playbacks that simulated two outsiders from different groups calling together, and socially congruous playbacks that simulated two outsiders from the same group calling together. Subjects responded more quickly to the incongruous playbacks, suggesting that they were aware that the callers belonged to two different groups. This study provides the first demonstration that animals can recognize associations between members of other groups under natural circumstances, and highlights the importance of considering how inter-group social selection pressures may influence the evolution of cognition.

Introduction

Why do animal taxa vary so markedly in their cognitive abilities? The *social intelligence hypothesis* posits that the demands of the social environment are the primary driving force

behind the evolution of complex cognition (Humphrey 1976). While abundant evidence supports a general positive association between social complexity and cognitive complexity (Dunbar and Shultz 2007; Emery et al. 2007), the ways in which different social selection pressures influence specific cognitive abilities are less clear. Understanding the evolution of a given cognitive ability requires investigating the full range of socio-ecological contexts in which the ability in question is used.

The social environment is not limited to the core social unit. In many taxa, successful mating (Lazaro-Perea 2001), territorial defense (Akçay et al. 2010), dispersal (Jungwirth et al. 2015), and cooperation with kin (Dickinson et al. 1996) frequently depend on knowledge about members of other social groups. However, while a number of studies have shown that animals can recognize territorial neighbors (Cheney and Seyfarth 1982; Stoddard 1996), more complex forms of social cognition have primarily been studied within the confines of a single social group (Seyfarth and Cheney 2015).

The ability to recognize and keep track of relationships and associations between others, known as *triadic awareness* or *third-party knowledge*, is critical for animals that engage in triadic social interactions such as alliances and coalitions (Seyfarth and Cheney 2015). Primates can recognize several types of relationships between third parties, including kinship (Cheney and Seyfarth 1980; Wittig et al. 2007), dominance rank (Cheney et al. 1995; Borgeaud et al. 2013), male-female sexual relationships (Bachmann and Kummer 1980; Crockford et al. 2007), and male-infant affiliative relationships (Kubenova et al. 2017). Observational evidence suggests that spotted hyenas *(Crocuta crocuta)* are aware of the dominance and kin relationships between other individuals (Engh et al. 2005) and that rooks *(Corvus frucilegus)* recognize one another's preferred social affiliates (Emery et al. 2007), while common ravens *(Corvus corax)* (Massen et

al. 2014) have been shown experimentally to recognize the dominance relationships between third parties.

Despite the prominence of triadic awareness within the social cognition literature, most studies have concentrated on whether animals have knowledge of the relationships between individuals within their own social group (*intra-group triadic awareness*). To my knowledge, the only unequivocal demonstration of *inter-group triadic awareness*, or knowledge about the relationships or associations between individuals in other groups, was in a single study of captive common ravens (Massen et al. 2014). Moreover, this study was conducted with two groups of only eight birds each housed in very close proximity, where individual ravens had extensive opportunity to repeatedly observe interactions among the same conspecifics. Ravens in the wild live in much larger social networks with fission-fusion dynamics, where learning the relationships among outsiders would presumably be more cognitively challenging (Braun and Bugnyar 2012). No previous study has examined whether animals are aware of the associations among individuals in other groups under natural conditions. Failing to investigate important cognitive abilities in the context of the full social environment risks ignoring major selection pressures on the evolution of social cognition. Thus, experimental studies of inter-group triadic awareness in an ecologically realistic context are needed to determine whether the benefits of this ability can outweigh the costs associated with its cognitive development.

I investigated inter-group triadic awareness in the acorn woodpecker (*Melanerpes formicivorus*), a cooperatively breeding bird with an unusually complex social system. Acorn woodpeckers in California live in family groups on stable, year-round territories with one to four joint-nesting females and one to eight co-breeding males (Koenig et al. 2016). Breeders of the same sex are nearly always close relatives and opposite sex breeders are unrelated; thus incest is

rare (Koenig et al. 1998). Moreover, availability of suitable breeding locations is limited, and thus individuals of both sexes are often forced to delay dispersal and remain in their natal territory for several years as nonbreeding helpers (Koenig et al. 2011). Group members cooperate to raise offspring in a single nest, store acorns within a "granary" tree, and defend resources from both conspecific and heterospecific intruders (MacRoberts and MacRoberts 1976; Koenig and Walters 2016).

Opportunities for helpers to breed typically only occur when all the breeders of the opposite sex have died or disappeared either in their natal territory (in which they would inherit breeder status) or in another territory (to which they would disperse). Breeders may occasionally leave their current group to fill a breeding vacancy in a different territory (Koenig and Mumme 1987). Competition to fill breeding vacancies is intense, with fights for vacancies sometimes starting within a few hours of the disappearance of a breeder and lasting for several days (Koenig 1981a; Hannon et al. 1985). Thus, reproductive success for both helper and breeder acorn woodpeckers often depends on being able to quickly identify when specific members of another group are missing, suggesting that individuals may routinely update knowledge about the members of other groups. When competing for breeding vacancies, acorn woodpeckers form coalitions with same-sex kin (Hannon et al. 1985), so an awareness of the relationships among individuals in other groups would likely help acorn woodpeckers assess the potential allies of their competitors. Acorn woodpeckers make regular forays to territories up to several kilometers away (mean foray distance = 2.47 km for males, 4.98 km for females) (Koenig et al. 1996), presumably allowing them to become familiar with a large number of individuals from other groups. I tested the hypothesis that acorn woodpeckers can determine whether two birds from outside the subject's own group belong to the same group as each other.

Material and Methods

(a) *Study site and population monitoring*

All data were collected at the Hastings Natural History Reservation in central coastal California, USA (36.379ºN, 121.567ºW). The acorn woodpecker population at this site has been the subject of a long-term study since 1968 (MacRoberts and MacRoberts 1976; Koenig 1981b), and >95% of the individuals are color-banded. Approximately 50 social groups are monitored, and a census is taken of each group approximately every 8-10 weeks. Subjects for the study described consisted of 15 adult acorn woodpeckers, each from a different social group. To control for sex and reproductive status, all subjects were female breeders (as opposed to non-breeding helpers). The subjects' groups had a mean size of 5.9 (range 3-12) individuals (including the subject) at the time of the study. Experimental trials were conducted from 17 March to 15 May 2016. Unless otherwise stated, values given are mean ± standard deviation.

(b) *Experimental design*

To test whether acorn woodpeckers could determine whether two individuals from outside their own group belonged to the same group as each other, I conducted a playback experiment using a violation-of-expectation paradigm, which is often used to test for triadic awareness (Cheney et al. 1995; Crockford et al. 2007). In this paradigm, subjects are presented with a playback simulating an anomalous social interaction between two callers, which should violate the subjects' expectations if and only if they recognize the relationship between the callers. Subjects are expected to respond more strongly to anomalous playbacks than to normal controls by reacting more quickly or looking toward the speaker for a longer period of time, among other behavioral responses (Proops et al. 2008). Acorn woodpeckers frequently produce greeting calls known as "*wakas"* in an overlapping chorus together with members of their own

group, but not with members of other groups (MacRoberts and MacRoberts 1976). I presented subjects with playback stimuli consisting of *waka* calls recorded separately from two different individuals at my study site, artificially overlapped to simulate two birds calling together as if they were members of the same group. In all cases, the callers were not from the same group as the subject. In half the trials, the callers were from the same group as each other (socially congruous control stimulus), and in the other half of trials the callers were from two different groups (socially incongruous test stimulus). Each subject received both a test stimulus and a control, spaced apart by two to five days (median = three) to reduce the chance of habituation. All observers were blind to stimulus identity until the trials were completed. A non-observer randomly assigned eight of the subjects to receive the test stimulus first and seven to receive the control stimulus first, and labeled the playback sound files with the appropriate subject's identification number.

I used a total of 13 *waka* calls recorded from 13 different callers to construct the playback stimuli, for a total of 12 unique test stimuli and five unique control stimuli. Each stimulus (unique combination of two overlapping calls) was used in one to five playback trials (1.76 ± 0.33 SE). Each individual call was used as a component in two to eight different stimuli (median = four) (Table 2.1).

Table 2.1. The identity of the individuals whose calls were presented to each subject, along with the distance between the subject's and the callers' territories (m), and the duration of the stimulus (s).

Group	Focal Female	Control Stimulus					Test Stimulus				
		Caller 1[1]	Caller 2	Caller Group	Caller Distance	Stimulus Duration	Caller 1	Caller 2	Caller 2 Group	Caller 2 Distance	Stimulus Duration
RE28[2]	F5700	M5511	F5103	JAIM	489.0	5.91	M5511	F4629	CAVI	594.8	5.91
JAIM	F5103	M4621	F4629	CAVI	107.4	4.27	M4621	F5700	RE28	489.0	5.22
PLQE	F4854	F5103	M5511	JAIM	101.8	6.17	F5103	M4621	CAVI	120.5	4.87
CAVI	F4629	M5511	F5103	JAIM	107.4	5.91	M5511	F4599	Y	285.7	5.91
KNOL	F5007	M5728[3]	F4599	Y	155.2	4.46	M5728	F5700	RE28	772.9	4.52
Y	F4599	F5300	M4889	LOLF	784.9	7.68	F5300	M4751	MLF2	595.3	6.77
ROBH	F5389	M5511	F5103	JAIM	904.1	5.91	M5511	F5700	RE28	1136.3	5.91
1500	F5126	M4889	F5300	LOLF	319.3	7.58	M4889	F5476	LHAY	615.6	5.90
1600	F5139	F5300	M4889	LOLF	299.5	7.68	F5300	M4751	MLF2	156.2	6.77
1800	F4261	F5300	M4889	LOLF	220.1	7.68	F5300	M4751	MLF2	177.7	6.77
MLF2	F4638	M5495	F5112	GATE	734.3	3.94	M5495	F4599	Y	595.3	5.04
LOLF	F5300	F5112	M5495	GATE	534.0	4.02	F5112	M4751	MLF2	200.3	3.78
LHAY	F5476	F5112	M5495	GATE	664.9	4.02	F5112	M4889	LOLF	531.4	6.88
WGAT	F5423	M5495	F5112	GATE	112.8	3.94	M5495	F5476	LHAY	639.3	5.89
GATE	F5112	M4889	F5300	LOLF	534.0	7.58	M4889	F5476	LHAY	664.9	5.90

[1] The first caller began a few syllables before the second caller (see Material and Methods: *Playback Stimuli*)

[2] F5700 was a breeder at RE28 during the study, but was a helper at PLQE a few months earlier, and her call was recorded while she was at PLQE. PLQE is much closer to the callers' territories than is RE28 (101.8 m from JAIM, 120.5 m from CAVI)

[3] The control stimulus to subject F5007 was the only stimulus in which the two callers (M5728 and F4599) were related. M5728 was the only caller that was a helper during the course of the playbacks

Each stimulus consisted of one call from a female bird and one call from a male bird, and in all cases but one control stimulus, the callers were both breeders. Because opposite-sex breeders in the same group are unrelated, this meant that the pair of callers in a given playback stimulus were unrelated to each other in all cases but one, even in the control stimuli where the two callers came from the same group.

For both test and control stimuli, callers were unrelated to the subject and the subject's current group members and had never lived in the same group as the subject or any of her current group members. This ensured the absence of an affiliative relationship between subjects and callers, which might have influenced the subjects' responses to the playbacks. Within the limits of this constraint, I presented each subject with calls recorded from the geographically closest group for which I had high-quality recordings to maximize the chance that subjects would be familiar with all the callers they were exposed to. The mean Euclidean distance between the granary of the subject's territory and the granary of the callers' territories was 430 ± 256 m (range 102–1136), which is well below the mean distance of extra-territorial forays in female acorn woodpeckers (4.98 km) (Koenig et al. 1996).

(c) *Playback protocol*

Before each playback trial, I placed a Yamaha PDX-11 speaker (Hamamatsu, Shizuoka, Japan) 1–1.5 m off the ground in a tree within the subject's territory, ~40 m away from another tree near the center of the territory (usually the granary tree), hereafter referred to as the "center tree." I always placed the speaker in the same location for each trial with a given subject, and trials were only conducted when the subject was sitting in the center tree. Because the anomalous "test" stimuli consisted of calls recorded from two different groups, it was impossible to broadcast both calls from the direction of the callers' actual territories and still have them

originate from a single location. Therefore, in both test and control trials the speaker was offset from the direction of either of the caller's territories so that the playback would originate from an unexpected direction. The angle between the speaker and either of the caller's territories (with the subject's center tree as the vertex) was ≥80º for all but one subject, for which the speaker was offset by ~45º from the territories of all the callers used in both the test stimulus and the control stimulus. This ensured that any differential responses to test vs. control stimuli would be due to recognition of the association between the callers and not whether the calls came from an unexpected direction.

Once the focal breeder female was located in the center tree, I played the appropriate playback file and began filming the focal female with a Panasonic SDR-H80 video camera (Kadoma, Osaka, Japan). The playback file contained one minute of background noise before the calls, which served as a pre-playback period. At the same time, a second observer watched the space between the center tree and the speaker tree and verbally noted on a digital recorder the times at which any acorn woodpecker flew from the center tree towards the speaker, the distance between the birds and the speaker, and when possible, the identity of the approaching individuals. I also placed a Sennheiser ME62 omnidirectional microphone (Wedemark, Germany) connected to a Roland R26 (Hamamatsu, Shizuoka, Japan) or a Fostex FR2 (Akishima City, Tokyo, Japan) digital recorder between the center tree and the speaker tree, to record any vocalizations produced by the subject's group (48 kHz sampling rate, 24 bits of amplitude resolution). Filming and behavioral observations continued for at least 10 min after the playback ended, but only the first three min of the playback and post-playback period were considered for analysis, as the woodpeckers almost always returned to baseline behavior in less than three min.

In groups with more than one breeder female, I selected the first breeder female that I saw during the first trial as the focal female for both trials with that group. Before conducting a playback trial, I attempted to ensure that no acorn woodpeckers were in the speaker tree or between the center tree and the speaker tree. If any acorn woodpeckers were spotted in the speaker tree or between the center tree and the speaker during the pre-playback period, then the trial was aborted and not restarted until the birds left the area. I also aborted a trial if a disturbance such as a predator, a loud vehicle, or a territorial intruder appeared during the pre-playback period, or if the focal bird went out of sight during the pre-playback period. If the playback vocalizations had already begun to play by the time I realized that the trial should have been aborted, then the trial was redone after a minimum of two days and the original trial was not used for analysis. I also redid trials if the focal bird did not stay in sight long enough to score most of the response variables of interest.

(d) *Recordings of playback stimuli*

I recorded all but two of the calls used for playback stimuli from January-July 2015 using a Sennheiser ME67 shotgun microphone (Wedemark, Germany) with a Rycote softie windscreen (Stroud, Gloucestershire, UK), and a Marantz PMD661 digital recorder (Kawasaki, Kanagawa, Japan) (48 kHz sampling rate, 16 bits of amplitude resolution). The remaining two calls were recorded from February-March 2016 using a Sennheiser ME62 omnidirectional microphone (Wedemark, Germany) with a windscreen and a Roland R26 digital recorder (Hamamatsu, Shizuoka, Japan) (48 kHz sampling rate, 24 bits of amplitude resolution). Calls were only used as playback stimuli when they were recorded from a single known individual with a relatively high signal to noise ratio.

(e) *Construction of playback stimuli*

I constructed the playback stimuli in Audacity® 2.1.1. The two calls recorded in 2016 at 24 bits of amplitude resolution were down-sampled to 16 bits using Raven Pro 1.5 (Cornell Lab of Ornithology, Ithaca, NY, USA) prior to processing. Playback stimuli consisted of a minute of background noise with a fade-in applied to the first 10 sec, followed by two overlapping calls, followed by 30 sec of background noise, followed by the same two overlapping calls, followed by a final 10 sec of background noise with a fade-out applied to all 10 sec. I included the initial minute of background noise so that there would not be a rapid onset of background noise at the same time as the onset of the first call. The calls were repeated because pilot data indicated that a single playback often failed to elicit a response, and because a previous study with acorn woodpeckers at Hastings used playback stimuli with a similar design (Yao 2008). While acorn woodpeckers most commonly produce single *waka* bouts, they sometimes produce two bouts in succession. All but two calls were filtered with a 200 Hz cutoff and a 6 dB roll off. Two calls had unusually loud low-frequency noise, so I used a 12 dB roll off with the same cutoff frequency. As the minimum frequency of *waka* calls is ~400 Hz, this filter is not expected to cause any noticeable distortion to the calls. The final playback stimuli each consisted of a stereo .WAV file (48 kHz sampling rate, 16 bits amplitude resolution) with one channel for each caller. Prior to overlapping the calls, I normalized each call to the same relative amplitude (-3 dB).

Waka calls consist of a variable number of repeated notes, with the first few and last few notes of each call typically being shorter and softer than the rest (MacRoberts and MacRoberts 1976). I overlapped the two calls in a given stimulus such that the first few “full-volume” notes of one call played before the second call began. This is consistent with natural *waka* choruses, in which one bird often begins calling alone and is then joined by one or more additional callers. I

overlapped the calls according to the following rule: if the first call has ≤6 full-volume notes, start the second call after the first full-volume note of the first call. If the first call has 7-10 full-volume notes, start the second call after the first two full-volume notes of the first call. If the first call has 11-14 full-volume notes, start the second call after the first three full-volume notes of the first call. I also ensured that at least some of the notes from each caller did not overlap to maximize the chance that the subject would be able to hear and recognize both callers.

(f) *Measuring degree of synchrony within playback stimuli*

As the degree of temporal synchrony between two overlapping callers could potentially contain information about the likelihood that they belong to the same group, I quantified the degree of synchrony of each of the playback stimuli. Call synchrony can be defined in different ways, so I measured four different metrics of synchrony for each playback stimulus: the average timing of the notes of the second caller relative to the notes of the first caller (Maynard et al. 2012), the consistency of the timing between the notes of the two callers, the proportion of the stimulus during which the callers were overlapping (*Proportion of Overlap*) (Maynard et al. 2012), and the *Lag Time* between the start of Caller 1 and the start of Caller 2 (Toth et al. 2012).

In order to measure the average relative timing and consistency of timing of the two overlapping callers within a playback stimulus, I first calculated the angular moment for each note of Caller 2 relative to the immediately preceding and following notes of Caller 1. *Angular Moment* was calculated according to the following equation

$$A = \frac{2\pi(X - R_1)}{R_2 - R_1}$$

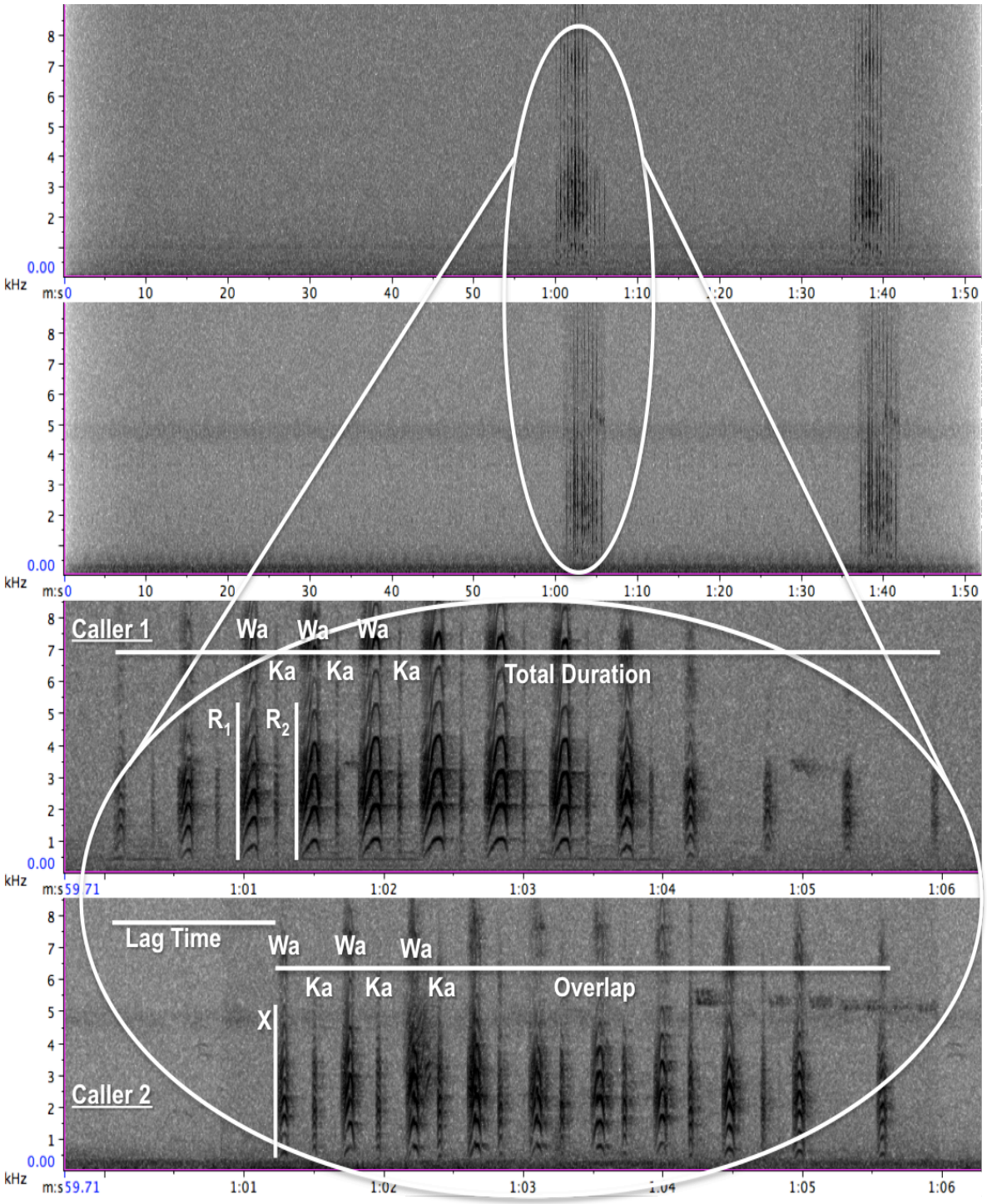
where X was the start time of a note from Caller 2, R_1 was the start time of the note from Caller 1 that immediately preceded X, and R_2 was the start time of the note from Caller 1 that immediately followed X (Maynard et al. 2012). This metric is expressed as an angle in radians,

where a value of 0 indicates that X equals R_1, π indicates that X is midway between R_1 and R_2, and 2π indicates that X equals R_2. I measured X, R_1, and R_2 by generating spectrograms of the playback stimuli in Raven Pro 1.5 (Hann window, window size=1226 samples, DFT=2048, overlap=90%), and using a band-limited energy detector to automatically detect the start and end times of each "*wa*" note, the longer of the two note types in a *waka* call. The detector was set to look for signals between 800 and 2000 Hz, with a minimum duration of 0.06 s and a maximum duration of 0.25 s, a minimum separation distance of 0.021 s, and minimum 70% occupancy above a signal-to-noise ratio threshold of 10. The signal-to-noise ratio was calculated using a sliding noise block of 2.001 s with a hop size of 0.489 s. I checked the results of the automatic detector by eye to ensure that each "*wa*" note was correctly detected. I calculated the circular mean of *Angular Moment* within each playback stimulus, which represented the degree to which the notes of the two callers tended to overlap or interleave on average (average timing of Caller 2 relative to Caller 1). I also calculated the standard deviation of the angular moments for each note of Caller 2, which represented the consistency of the timing of Caller 2's notes relative to Caller 1's notes.

I calculated *Proportion of Overlap* as the duration of time during which Caller 1 and Caller 2 overlapped divided by the total duration of the playback stimulus. In many duetting species, a greater degree of overlap between phrases indicates a less synchronization, because the participants sing phrases in turn to give the impression of one continuous song (Cuthbert and Mennill 2007; Elie et al. 2010). However, because *waka* choruses usually consist of a single "phrase" uttered by each participant, it seemed more appropriate to consider higher degree of overlap as indicating greater synchrony. *Lag Time* was the difference between the start time of

Caller 2 and the start time of Caller 1, with a smaller *Lag Time* indicating a more synchronous chorus (Toth et al. 2012) (Figure 2.1).

Figure 2.1. Spectrogram of a playback stimulus (Hann window, 1135 samples/window, DFT=2048 samples, 80% overlap) with illustrations of how each metric of call synchrony was measured. The top two bars display the full length of the playback file with an initial minute of background noise with a 10 sec fade-in, then the first set of overlapping calls, then thirty seconds of background noise, the same overlapping calls repeated, and 10 final sec of background noise with a fade-out applied to all 10 sec. The bottom two bars zoom in on the first set of overlapping calls. Each *waka* call consists of a series of alternating "*wa*" and "*ka*" notes, indicated with white labels. The white lines and labels illustrate how each of the metrics of call synchrony (*Angular Moment*, *Proportion of Overlap*, and *Lag Time*) was measured. *Angular Moment* was defined as $2\pi*(X-R_1)/(R_2-R_1)$, and was calculated for each individual "*wa*" note of Caller 2. *Proportion of Overlap* was calculated as overlap/total duration.


kHz
m:s
Caller 1
Wa
Wa
Wa
Ka
Ka
Ka
Total Duration
R1
R2
Lag Time
Wa
Wa
Wa
Ka
Ka
Ka
Overlap
X
Caller 2

(g) *Characterizing amplitude of playback system*

To determine whether the playbacks were at a naturalistic volume, I measured the amplitude of 26 *waka* calls produced by a captive adult male acorn woodpecker using the SoundMeter 8.4.3 iPhone app (Faber Acoustical, Lehi, UT, USA) with the internal microphone on an iPhone 6. The SoundMeter app was set to flat (Z) weighting, fast response, and Lp level. I held the iPhone 1 m away from the bird with the iPhone's internal microphone pointed towards him and recorded the peak sound pressure level whenever he vocalized. These measurements were not conducted in an anechoic chamber. The amplitude of the *waka* calls that I measured from this individual ranged from 77.7 to 100.1 dB re 20 µPa at 1 m, with a mean value of 92.5 ± 5.4 dB.

After completing the experiment, I also used the SoundMeter app with the same iPhone 6 to characterize the amplitude of the playback system. I placed the speaker in an anechoic chamber 1 m from the iPhone. Both devices were placed on isolation pads on top of pedestals such that the center of the speaker was at the same height as the iPhone, and the speaker and iPhone microphone were facing each other. The SoundMeter app was set to a flat (Z) weighting, fast response, and Lp level, and the speaker was set to maximum gain (as in the original playback trials). Using the same iPod Touch that I used during the original playback trials (set to maximum gain, as in the original trials), I played six of the original playback stimuli three times each and recorded the peak sound pressure level. The mean peak sound pressure level of the playback stimuli was 100.1 ± 1.3 dB re 20µPa at 1 m. Thus, the amplitude of the playbacks was likely near the upper end of the range of natural *waka* calls.

(h) *Measuring response to playback*

Using the video and audio recordings of each playback trial, I measured the latency to the focal female's first "directional" flight, defined as flying up to a higher vantage point or flying closer to the speaker. In addition, I measured the following five response variables but did not include them in the analysis because they were highly correlated (|Pearson's r|≥0.7) with latency to first directional flight: latency to the focal female's initial flight in any direction, latency to her first "reaction" ("directional" flight or vocalization, whichever came first), latency to her closest approach to the speaker, distance of her closest approach to the speaker, and direction of her initial flight on an ordinal scale (-1=away from the speaker, 0=parallel to speaker with no height gain, 1=to a higher vantage point without getting closer or further from the speaker, 2=towards the speaker).

To assess the response of other group members, I measured the latency to the first vocalization by any group member, the distance of the closest approach to the speaker by any bird other than the focal female, the change in the group's *waka* call rate between the minute immediately preceding the start of the playback and the minute immediately following the start of the playback, and the proportion of the subject's group members that approached the speaker. I also measured the following variables that were highly correlated with other response variables and therefore excluded from analysis: latency to the first flight from the center tree towards the speaker by any bird other than the focal female, latency to the closest approach to the speaker by any bird other than the focal female, and the number of birds that gathered around the focal female following the playback. For all latency response variables, if the behavior of interest did not occur within the first three min after the start of the playback, the latency was assigned the

maximum possible value of 180 sec and marked as 'censored'. All response variables and their definitions are described in Table 2.2.

Table 2.2. Definitions of the response variables measured during each playback trial that were not highly correlated (|Pearson's r|<0.7) with any other response variables included in the analysis.

Response Variable	Definition
Focal Bird Latency to First "Directional" Flight	Time in sec from the start of the first call in the playback file until the focal female flew up and landed at a higher vantage point or flew closer to the speaker (censored after 3 min)
Latency to First Call by Any Individual	Time in sec from the start of the first call in the playback file until the first vocalization of any call type by any member of the group (censored after 3 min)
Non-focal Birds Closest Approach Distance	The closest distance between any bird *other than* the focal female and the speaker at any point in time within the 3 min after the start of the first call in the playback file (closest approach distance by the focal female was excluded from analysis because of correlation with other response variables)
Proportion of Group Members Approaching	Proportion of group members that flew out of the center tree towards the speaker within 3 min after the start of the first call in the playback file
Change in Group Waka Call Rate	Number of waka calls produced by any group member in the first min after the start of the first call in the playback file, minus the number of waka calls produced in the min preceding the first call in the playback file

(i) *Predictions*

As both the test and control stimuli represented a territorial intrusion by outside birds, I expected some degree of aggressive response to both conditions. However, I predicted that if

acorn woodpeckers can determine whether two individuals from outside their own group belong to the same group as each other, then they should react more strongly to test playbacks than to controls, reflecting a "violation of expectation" caused by the socially incongruous test stimuli, and also potentially reflecting the higher threat level associated with a simultaneous intrusion by two groups instead of only one (Cheney et al. 1995; Radford 2003). Specifically, I predicted that subjects would exhibit shorter response latencies and approach the speaker more closely (smaller approach distance) in the test condition than in the control condition. I also predicted that a greater proportion of the subject's group would approach the speaker in the test condition than in the control condition, and that the group's *waka* call rate would increase more (from pre-playback to post-playback period) after test playbacks than after control playbacks.

(j) *Statistical analyses*

I conducted all statistical analyses in R 3.4.3 (R Core Team 2017). I constructed separate models for each response variable to facilitate interpretation of the results. I used mixed-effects Cox proportional hazards regression models in the R package "*coxme*" (Therneau 2018) to analyze the latency to the focal female's first directional flight and the latency to the first vocalization by any group member. I used linear mixed-effects models in the R package "*lme4*" (Bates et al. 2015) to analyze the closest approach distance by any bird other than the focal female and the change in *waka* call rate before and after the playback. As the residuals were not normally distributed for the change in *waka* call rate, I square root transformed this response variable. I used a generalized linear mixed model with a binomial distribution in the R package "*lme4*" to analyze the proportion of the subject's group that approached the speaker.

In each model, I included *Treatment* (test or control) as a fixed effect, and *Subject ID* and *Stimulus ID* (unique combination of two callers) as random effects. When constructing playback

stimuli, I was unable to hold constant the duration of the overlapping chorus, the mean distance between the two callers' territories and the territory of the subject that heard the playback (which could potentially affect the subject's familiarity with the caller), and the degree of synchrony between the two overlapping calls. To ensure that these factors did not explain any difference in response to test and control stimuli, I re-ran the models with *Stimulus Duration, Mean Caller-Subject Territorial Distance*, *Standard Deviation of Angular Moment*, *Proportion of Overlap*, and *Lag Time* added as covariates. Before adding these variables to the models, I transformed them by subtracted the midpoint and dividing by ½ the range, so that each variable would be on a scale from -1 to +1. I could not include *Circular Mean of Angular Moment* in the models because of the circular nature of this variable, so I conducted a Watson's two-sample test to determine if *Circular Mean of Angular Moment* differed between the test and control stimuli. Alpha level was set to 0.05 for all tests, and all tests were two-tailed.

Results

With three factors, five covariates, and a sample size of 15 individuals, the full models were likely over-parameterized, and the addition of the five covariates did not change the significance of *Treatment* in any model. Therefore, I present the results of the more parsimonious models in both the main text and in Table 2.3 and include the results of the full models in Table 2.4 only.

Table 2.3. Summary of each reduced model (no fixed effect covariates). Parameter estimates (coefficients), test-statistics, and *P*-values are displayed for *Treatment*. The variance component for each random effect (*Individual ID* and *Stimulus ID*) is also displayed. * = Significant at 5% level.

Response Variable	Model Type	N	Treatment			Indiv. ID Variance	Stim. ID Variance
			Coef.	Test Statistic	*P*-val		
Focal Latency to First Directional Flight	Mixed-Effects Cox Model	15	1.20	χ^2=7.91	0.005*	1.19	0.00
Latency to First Call by Any Individual	Mixed-Effects Cox Model	15	-0.20	χ^2=0.32	0.57	0.09	0.00
Non-focal Birds Closest Approach Distance	Linear Mixed Model	15	2.32	$F_{1,14.2}$= 0.16	0.69	52.29	0.00
Change in Group Waka Call Rate	Linear Mixed Model	15	-0.04	$F_{1,9.7}$= 0.01	0.92	0.08	0.43
Prop. of Birds Approaching	Binomial GLMM	14	0.32	χ^2=0.41	0.52	0.32	0.13

Table 2.4. Summary of each full model with all covariates included. Parameter estimates (coefficients), test-statistics, and *P*-values are displayed for all fixed effects, and the variance components are displayed for the two random effects (*Individual ID*, *Stimulus ID*). * = Significant at 5% level.

Response Variable	Model Type	N	Treatment	StDev Angular Moment	Prop. Overlap	Lag Time	Stimulus Duration	Inter-territory Distance	Random Effects
Focal Latency to First Directional Flight	Mixed-Effects Cox Model	15	Coef=1.46 χ^2=7.91 *P*=0.005*	Coef=0.02 χ^2=0.51 *P*=0.48	Coef=0.02 χ^2=0.55 *P*=0.46	Coef=1.02 χ^2=0.68 *P*=0.41	Coef=-1.38 χ^2=8.02 *P*=0.005*	Coef=0.32 χ^2=0.06 *P*=0.80	0.00, 0.00
Latency to First Call by Any Individual	Mixed-Effects Cox Model	15	Coef=-0.79 χ^2=0.32 *P*=0.57	Coef=-0.56 χ^2=1.58 *P*=0.21	Coef=-0.78 χ^2=4.31 *P*=0.038*	Coef=-0.42 χ^2=0.06 *P*=0.81	Coef=0.35 χ^2=0.83 *P*=0.36	Coef=0.07 χ^2=0.71 *P*=0.40	0.00, 0.00
Non-focal Birds Closest Approach Distance	Linear Mixed Model	15	Coef=1.29 $F_{1,1.5}$=0.04 *P*=0.87	Coef=4.45 $F_{1,3.3}$=0.57 *P*=0.50	Coef=-4.25 $F_{1,10.1}$=0.42 *P*=0.53	Coef=-3.95 $F_{1,11.5}$=0.35 *P*=0.56	Coef=2.43 $F_{1,1.6}$=0.14 *P*=0.75	Coef=-5.02 $F_{1,13.5}$=0.54 *P*=0.47	72.73, 4.07
Change in Group Waka Call Rate	Linear Mixed Model	15	Coef=-0.13 $F_{1,8.6}$=0.09 *P*=0.78	Coef=-0.09 $F_{1,13.6}$=0.08 *P*=0.79	Coef=-0.59 $F_{1,13.5}$=2.58 *P*=0.13	Coef=0.30 $F_{1,12.9}$=0.78 *P*=0.39	Coef=-0.13 $F_{1,10.0}$=0.09 *P*=0.77	Coef=-0.02 $F_{1,10.1}$=0.01 *P*=0.94	0.18, 0.46
Prop. of Birds Approaching	Binomial GLMM	14	Coef=0.54 χ^2=2.09 *P*=0.15	Coef=-0.29 χ^2=0.79 *P*=0.37	Coef=-0.22 χ^2=0.46 *P*=0.50	Coef=1.17 χ^2=10.59 *P*=0.001*	Coef=-0.88 χ^2=6.97 *P*=0.008*	Coef=0.02 χ^2=0.00 *P*=0.94	0.00, 0.00

(a) *Response of the focal breeder female*

The latency to the focal female's first directional flight was significantly shorter (faster response) in the test condition than in the control condition (Cox regression, N=15, $\hat{\beta}$=1.2, χ^2=7.91, P=0.005) (Figure 2.2).

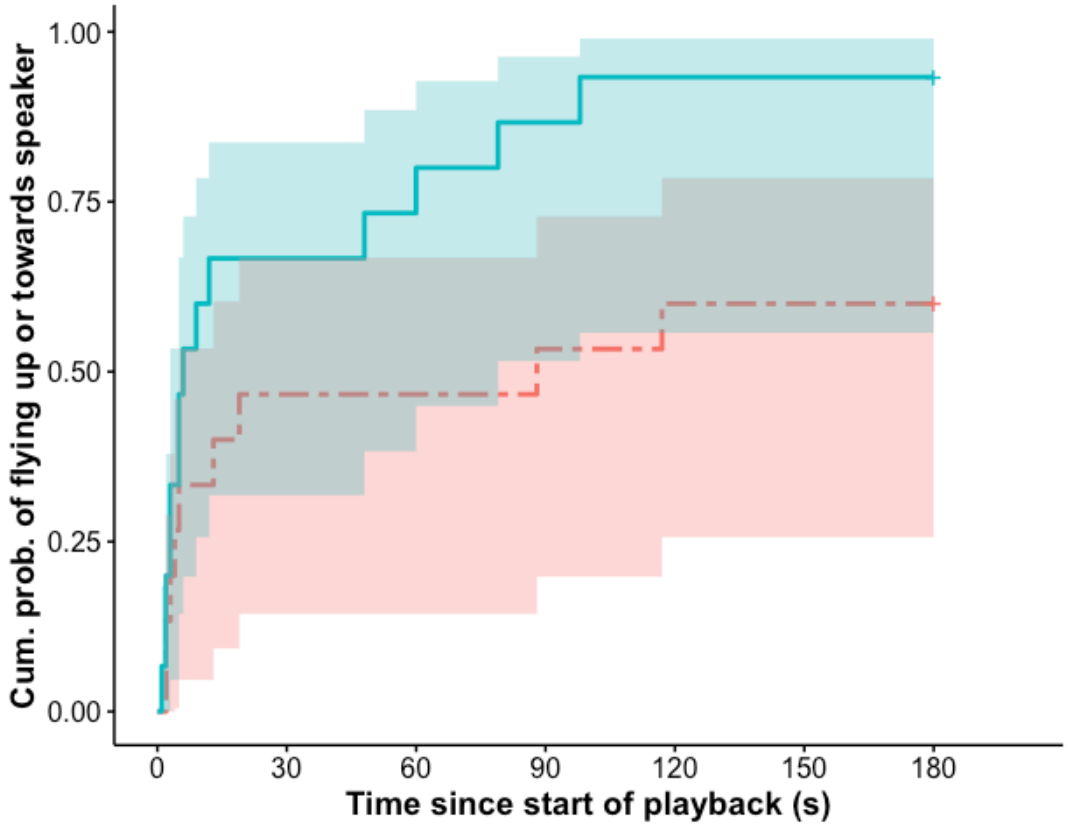


Figure 2.2. Kaplan-Meier survival curves for the latency to the focal female's first directional flight in socially congruous control playbacks (red dashed line) vs. socially incongruous test playbacks (blue solid line) (N=15). The lines indicate the cumulative probability that a "directional" flight (flight to a higher vantage point or towards speaker) will occur by a given point in time. The shaded areas around each line represent 95% confidence intervals. The curve for the test treatment rises significantly faster than the curve for the control, indicating a faster response time to the socially incongruous test stimuli (P=0.005).

(b) *Responses of other birds*

Treatment was not significantly related to the latency to the first vocalization by any group member (Cox regression, N=15, $\hat{\beta}$=-0.20, χ^2=0.32, P=0.57). It was also not significantly related to the closest approach distance by a non-focal bird (LMM, $\hat{\beta}$=2.32, $F_{1,14.2}$=0.16, P=0.69), the change in the group's *waka* call rate (LMM, N=15, $\hat{\beta}$=-0.04, $F_{1,9.7}$=0.010, P=0.92), or the proportion of group members that approached the speaker (Binomial GLM, N=14, $\hat{\beta}$=0.32, χ^2=0.41, P=0.52).

(c) *Circular mean of angular moment*

Test and control stimuli did not differ in the degree to which the notes of the two callers overlapped (Watson's two-sample test, U^2=0.095, P>0.10).

Discussion

These results indicate that breeder female acorn woodpeckers are capable of determining whether or not two individuals from outside their own group belong to the same group as each other. Many territorial animals can indirectly assess the group membership of their neighbors by associating individual neighbors with particular territories (Cheney and Seyfarth 1982; Akçay et al. 2010). However, because I broadcast my playbacks from a different direction than the territories of the callers, the subjects' knowledge of the group membership of others did not depend on associating callers with particular locations. Instead, my results suggest that the subjects were able to infer the association (shared group membership) between callers from other groups.

Many songbirds can glean information about territorial neighbors by eavesdropping on their vocal interactions with others, but in most cases, the birds could be cueing in on the aggressiveness or win-loss record of each individual conspecific in isolation, without necessarily

knowing anything about dyadic relationships (Akçay et al. 2010; Toth et al. 2012). Great tits *(Parus major)* (Peake et al. 2002), pinyon jays *(Gymnorhinus cyanocephalus)* (Paz-y-Miño C et al. 2004), and Burton's mouthbrooders *(Astatotilapia burtoni)* (Grosenick et al. 2007) were able to learn the dominance relationship between two conspecifics after witnessing one to seven interactions between them, independent of the absolute aggressiveness or win-loss record of each individual. In contrast to my study, however, these animals were provided with information about the relationships between others by the experimenters, so it is not clear whether they were aware of third-party relationships under natural conditions. Nonetheless, the rapidity with which all three of these species learned the relative dominance ranks of unfamiliar individuals suggests that many species may have the capacity to learn the relationships between members of other groups, despite the fact that interaction with non-group members is less frequent than interaction with members of one's own group. Thus, inter-group triadic awareness may be more common than current research suggests.

There are at least two mechanisms by which the acorn woodpeckers in my study could have determined whether two given callers were from the same group. One possibility is that *waka* calls carry an acoustic signature of group identity (Price 1999). If such a group signature exists, acorn woodpeckers could determine whether two conspecifics belong to the same group by comparing their calls and assessing whether the signatures match, without necessarily recognizing either bird or having any prior knowledge about their association.

The existence of acoustic group signatures, however, is unlikely in acorn woodpeckers. A previous study examining the acoustic structure of *waka* calls in the acorn woodpeckers at my study site found that while *wakas* were individually distinct, there was no evidence of group signatures (Yao 2008). This study also found that individuals treated playbacks of their own

waka calls like the *waka* calls of outsiders, rather than like the *waka* calls of fellow group members, which strongly suggests that recognition is not based on a group signature (Yao 2008). Moreover, over 70% of individuals that eventually attain breeding status do so via dispersal to a different group (Koenig et al. 2000) with a minority of dispersers changing groups twice or more during their lifetime, and opposite-sex breeders within the same group virtually always come from different natal territories (Koenig et al. 1998). Consequently, any acoustic signature shared by all members of a group could only be maintained via open-ended vocal production learning. While vocal learning has not been studied in any woodpecker species, it is currently only known in three avian orders (Passeriformes, Apodiformes, and Psittaciformes), and open-ended vocal learning is relatively rare even in taxa where vocal learning exists (Nottebohm 1972; Baptista and Schuchmann 1989). My results cannot be explained by the existence of genetically determined kin signatures, because in all but one case, the playback stimuli (both test and control) consisted of the calls of one male breeder and one female breeder that were unrelated (Table 2.1). Although shared group membership could potentially be signaled by the degree of synchronicity of a *waka* chorus, this is unlikely to explain my results because none of the metrics of synchronicity that I measured were significantly related to response latency.

I believe the more plausible explanation for my results is that acorn woodpeckers recognize the calls of individual members of other groups, and can integrate this information with knowledge about which group each caller belongs to in order to infer the association between two callers. This mechanism implies a more complex mental representation of the associations between third parties than the group signature hypothesis. Regardless of the underlying cognitive mechanism, however, this study demonstrates that wild acorn woodpeckers

recognize associations between members of other social groups without being artificially primed with information about those associations.

Acorn woodpecker knowledge of the group membership of others most likely extends beyond immediate neighbors. Out of the 15 subjects in this study, only three were presented exclusively with calls from territories that were immediately adjacent to their own. The remainder received at least one playback involving calls recorded from a non-adjacent territory, and for nine subjects both the test and control stimuli contained at least one call from a non-adjacent territory. Thus, it is likely that breeder female acorn woodpeckers can recognize the group membership of at least some of the birds in groups two or more territories away from their own. Furthermore, given the regular long-distance forays made by acorn woodpeckers (Koenig et al. 1996), it is possible that they recognize birds much further away than two territories. Additional work is necessary to determine the geographic extent of vocal recognition and triadic awareness in this species.

I originally focused on breeder females, as opposed to another sex or reproductive class, for practical reasons unrelated to this experiment. Nonetheless, inter-group social knowledge may be more important for female acorn woodpeckers, because females are less likely than males to inherit a breeding position in their natal territory, and are thus more dependent on being able to identify breeding vacancies in other groups (Hannon et al. 1985). Inter-group social information might also be more relevant to helpers than to breeders, because helpers are more likely to need to disperse.

Acorn woodpeckers have a social system in which knowledge about the associations among members of other groups could be particularly beneficial, both for identifying breeding opportunities and for predicting the size and membership of rival coalitions. I have found

evidence that at least breeder female acorn woodpeckers can determine whether two individuals from other groups have an associative relationship. This finding supports the prediction of the social intelligence hypothesis that a species' cognitive abilities will be adapted to its social environment. This study also highlights the importance of accounting for social selection pressures external to the core social group when investigating the evolution of social cognition in general. Future work on social cognition should consider the cognitive demands of inter-group social interactions as well as intra-group interactions.

CHAPTER 3

FEMALE ACORN WOODPECKERS RECOGNIZE RELATIONSHIPS BETWEEN THIRD PARTIES THAT HAVE NOT LIVED TOGETHER FOR YEARS

Abstract

Triadic awareness, or knowledge of the relationships between others, is essential to navigating complex social interactions. While many animals maintain relationships with former group members post-dispersal, recognizing such relationships between others is more cognitively challenging than recognizing relationships between members of a single group, because there is less opportunity to observe interactions between individuals that do not live together. I presented acorn woodpeckers (*Melanerpes formicivorus*) with five playback stimuli, each consisting of a simulated chorus between two different individuals, which only occurs naturally between social affiliates. Subjects were expected to respond rapidly if they perceived the pair of callers as not having an affiliative relationship, and less rapidly if they perceived the callers as having an affiliative relationship. Females responded rapidly to a pair of callers that never lived together (whether callers were genetically related or not), and to a pair of genetically related callers that last lived together after the subject fledged but ≥1 year before the experiment. They responded less rapidly to genetically related callers that lived together before the subject hatched and to genetically related callers that lived together at the time of the experiment. This suggests that female acorn woodpeckers can infer the existence of relationships between conspecifics that live in separate groups by observing them interact with one another post-dispersal. This study provides the first experimental evidence that animals can recognize relationships between third parties that no longer live together, and emphasizes the potential importance of extended social networks in shaping the evolution of intelligence.

Chapter 3: Recognition of relationships between individuals that no longer live together

Introduction

The ability to recognize relationships between other individuals, known as triadic awareness or third-party knowledge, is vital for navigating complex social interactions such as alliances and coalitions (Seyfarth and Cheney 2015). Triadic awareness has been most extensively studied in primates (Cheney and Seyfarth 1980; Cheney et al. 1995; Perry et al. 2004; Crockford et al. 2007; Kubenova et al. 2017), and has also been documented in a variety of other vertebrates, including carnivores (Engh et al. 2005), cichlid fish (Grosenick et al. 2007), and birds (Massen et al. 2014). However, the vast majority of work has focused narrowly on knowledge of third-party relationships between current members of the subject's own social group, potentially ignoring other important social contexts in which triadic awareness is used. Many animals regularly interact with other social groups, so an ability to recognize third-party relationships among individuals in other groups is likely beneficial. For example, family groups of African elephants (*Loxodonta africana*) maintain close social bonds with other family groups, so knowledge about members of other groups could be important for navigating social interactions when bonded groups meet (Moss and Poole 1983; Wittemyer et al. 2005). Similarly, the cichlid fish *Neolamprogus pulcher* visits other groups prior to dispersal, and knowledge about the social relationships among members of the target group could improve the chances of successful integration (Jungwirth et al. 2015).

Recognition of the relationships among members of other groups could take two different forms. In one scenario, individuals A and B have a relationship and live in the same group as each other but a different group from individual C, and C recognizes the relationship between A and B. In the other scenario, A and B live in separate groups but still maintain a relationship, and individual C, who lives a third group, is able to recognize the relationship between A and B. As

many animals maintain lifelong relationships with former group members by visiting them post-dispersal (Dickinson et al. 1996; Boeckle and Bugnyar 2012), an ability to recognize such "inter-group" relationships among third parties that no longer live together could be particularly important. However, such an ability would likely be more cognitively demanding than recognition of the relationships between third parties that currently live together, because individuals that maintain a "long-distance" relationship across group boundaries presumably interact less frequently than individuals that live together, and therefore provide fewer opportunities for other individuals to learn about their relationship.

Two recent studies have demonstrated that some animals have the capacity to recognize relationships among individuals in other social groups. When two mixed-sex groups of common ravens (*Corvus corax*) were housed in adjacent cages, the males recognized simulated reversals in dominance status between members of the neighboring group (Massen et al. 2014). Similarly, wild female acorn woodpeckers (*Melanerpes formicivorus*) were able to determine whether two individuals from outside their own social group belonged to the same group as each other (Pardo et al. 2018). However, no prior study has investigated whether animals can recognize relationships between third parties that no longer live together.

Acorn woodpeckers in California live in cooperatively breeding family groups with one to four joint-nesting females, one to eight cobreeding males, and up to 10 nonbreeding helpers of either sex, which are the adult offspring of the breeders (Koenig 1981b). Co-breeders of the same sex are close relatives, but opposite sex breeders are unrelated (Haydock et al. 2001). Suitable breeding territories are limited (Koenig et al. 2011), and thus when all the breeders of a particular sex die or disappear from an existing group, helpers from other groups compete to fill the vacancy in dramatic "power struggles" (Koenig 1981a; Hannon et al. 1985). To improve their

chances of competing successfully, helpers form coalitions with same-sex relatives, and coalition members often share the breeding position as co-breeders upon winning the power struggle (Hannon et al. 1985). Both males and females make frequent extraterritorial forays to other groups, which may help them become familiar with a large number of individuals from other groups and identify breeding vacancies (Koenig et al. 1996).

Acorn woodpeckers appear to maintain relationships with, or at least remember, their former group members even years after they have dispersed. Individuals sometimes return to their natal group years after dispersal and former group-mates that currently live in separate groups sometimes form a coalition during power struggles (N. Hagemeyer, pers. comm.). If acorn woodpeckers can recognize such long-term, cross-group relationships between third parties, it could potentially help them assess the number of allies that a rival could recruit during a power struggle. However, while acorn woodpeckers have been shown to recognize the association between members of another group that currently live with each other (Pardo et al. 2018), it is unknown if they can also recognize third-party relationships between individuals that used to live together but no longer do so.

I investigated whether male and female acorn woodpeckers can recognize kin relationships between members of other groups that do not live together, and if so, by what mechanism. Specifically, I tested the following hypotheses: H_0: Acorn woodpeckers only recognize kin relationships between members of other groups that currently live together; H_1: Acorn woodpeckers can determine if two individuals living in separate groups are related to one another via a vocal signature of kinship; H_2: Acorn woodpeckers can recognize relationships between members of other groups that no longer live together only if they observed those individuals living together in the past; H_3: Acorn woodpeckers can recognize relationships

between members of other groups that no longer live together by observing the individuals in question visit one another after those individuals dispersed into separate groups.

Based on the results of Pardo et al. (2018), I expected subjects to respond more strongly (shorter latency and/or closer approach) to playback of an overlapping chorus between callers that are unrelated to each other and never lived in the same group compared to playback of an overlapping chorus between callers that are related to each other and lived in the same group at the time of the experiment. This is because acorn woodpeckers typically only produce overlapping choruses with their social affiliates, so subjects should perceive a simulated chorus between two unaffiliated individuals as highly anomalous. My predictions for how subjects would respond to a pair of callers that do not currently live together but have some sort of relationship differed under each of the hypotheses (Figure 3.1).

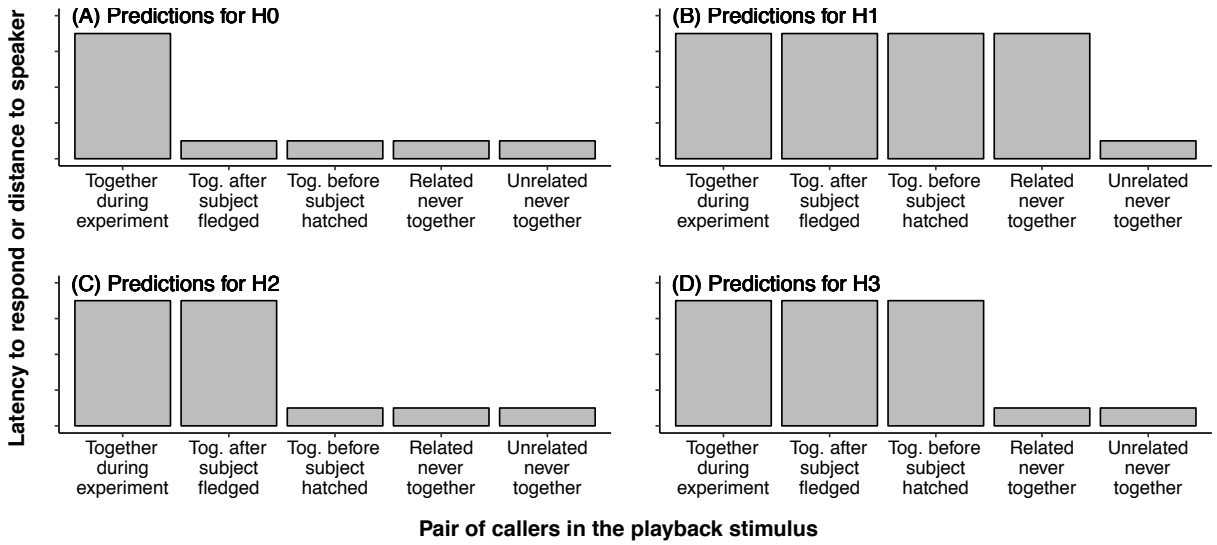


Figure 3.1. Predictions under each hypothesis. The y-axis indicates the relative strength of response predicted for each treatment under each hypothesis, with higher values of Y indicating a weaker response (slower latency to respond or more distant approach to the speaker). The four hypothesis are as follows (clockwise from top left): H_0 = subjects only recognize relationships between individuals that currently live together, H_1 = subjects use vocal kin signature to recognize kin relationships between others, H_2 = subjects can recognize relationships between callers that no longer live together if the subjects observed the callers living together in the past, H_3 = subjects recognize relationships between callers that no longer live together by observing the callers visit one another post-dispersal.

If acorn woodpeckers cannot recognize relationships between third parties that no longer live together (H_0), subjects should respond similarly to all callers that do not currently live in the same group, regardless of whether the callers are related to each other or whether the callers ever lived together in the past. If acorn woodpeckers use a vocal kin signature to determine whether birds from other groups are related to each other (H_1), then they should react less strongly to a pair of birds that are related to each other than to a pair of birds that are unrelated. If acorn woodpeckers recognize relationships between third parties that no longer live together by remembering that the individuals in question used to live together (H_2), then subjects should respond less strongly to a pair of callers that are related to each other and last lived together after

the subject fledged than to a pair of callers that are unrelated to each other and never lived in the same group. However, they should not differentiate between callers that are unrelated to each other and have never lived together and callers that are related to each other but never lived together or last lived together before the subject hatched.

Finally, if acorn woodpeckers recognize relationships between individuals that no longer live together by observing the individuals in question interacting *after* they dispersed to separate groups (H_3), then subjects should respond less strongly to a pair of callers that lived in the same group either before or after the subject fledged compared to a pair of callers that never lived in the same group, but should not differentiate between unrelated and related callers that never lived in the same group.

I also predicted that if any sex difference exists in triadic awareness, females should be better able or more motivated than males to keep track of relationships between members of other groups, given that females are more likely to disperse and compete for breeding vacancies in other groups (Hannon et al. 1985; Koenig et al. 1996). If females are better than males at identifying relationships between individuals in other groups, then females should better differentiate among treatments than do males.

Material and Methods

(a) *Study site and population monitoring*

I conducted my work at Hastings Natural History Reservation in central coastal California, where the acorn woodpecker population has been the subject of a long-term study since 1968 and >95% of the individuals are color-banded (MacRoberts and MacRoberts 1976; Koenig 1981b). Approximately 50 social groups are monitored, and a census is taken of each group approximately every 8-10 weeks. Subjects for this experiment were 26 wild adult acorn

woodpeckers, including nine breeder females, five helper females, seven breeder males, and five helper males from 18 social groups. Experimental trials were conducted from 19 Jul to 27 Nov 2017 and from 6 May to 8 Jul 2018.

(b) *Experimental design*

I conducted a playback experiment with a violation-of-expectation paradigm similar to Chapter 2 (Pardo et al. 2018). Woodpeckers were presented with playback stimuli consisting of *waka* calls recorded from two different individuals, overlapped artificially to simulate two birds calling simultaneously. *Waka* calls are individually-specific, affiliative vocalizations that are frequently given in an overlapping chorus between two or more members of the same group, but rarely given between individuals with no affiliative relationship (MacRoberts and MacRoberts 1976; Yao 2008). If the two overlapping callers in a playback stimulus belong to different groups and have no affiliative relationship, then the playback stimulus should violate the expectations of the subjects and they should respond more strongly by approaching the speaker more rapidly and more closely. Conversely, if the two overlapping callers in a playback stimulus have an affiliative relationship of which the subjects are aware, then the subjects should respond less strongly (Pardo et al. 2018). I presented subjects with playbacks from the following five treatment categories:

Related callers/currently live together: two related callers that lived in the same group at the time of the experiment.

Related callers/last together after subject fledged: two related callers that formerly lived in the same group but ceased to do so due to dispersal or death of one individual ≥1 year prior to the experiment and after the subject had fledged. Thus, the subject would have had the opportunity to observe the callers living together in the past.

Related callers/last together before subject hatched: two related callers that formerly lived in the same group, dispersed into separate groups before the subject hatched and ≥1 year prior to the experiment, and were both confirmed to be alive after the subject had fledged. Thus, the subject would not have had the opportunity to observe the callers living together, but could have observed them visiting one another post-dispersal.

Related callers/ never lived together: two related callers that never lived in the same group and were both confirmed to be alive after the subject fledged.

Unrelated callers/never lived together (control): two unrelated callers that never lived in the same group and were both confirmed to be alive after the subject fledged.

In all cases, the callers used in a given playback stimulus were unrelated to the subject and had never lived in the same group as the subject. Playback stimuli only contained recordings from callers of the same sex as the subject. I did this to increase the likelihood that the subjects would respond to the playbacks; all the playbacks simulated territorial intrusions, and acorn woodpeckers are more responsive to same-sex intruders (Hannon et al. 1985).

Nine female subjects and 10 male subjects received all five treatments. The remaining five females and two males each received all treatments except "Related callers/last together before subject hatched" due to a lack of appropriate recordings. In two cases, the treatment "Related callers/currently live together" included the call of an individual that apparently died shortly before the experiment, and this was not realized until later when censuses were tabulated, so I excluded these two trials from analysis. The order of presentation to a given subject was balanced using an incomplete Latin square design, and playbacks to the same group were spaced apart by 2-48 days (median = four days) to avoid habituation. Playbacks to groups within 250 m of one another were also spaced apart by at least two days.

(c) *Selection of calls for playback stimuli*

All calls used as playback stimuli were recorded from known individuals in the study population between January 2015 and July 2017. Recordings were made with a Sennheiser ME67 or ME62 microphone and a Marantz PMD661, Fostex FR2, or Roland R26 digital recorder (48 kHz sampling rate, 16 or 24 bit resolution). I used 10 unique pairs of callers as "Related callers/currently live together" stimuli, 15 as "Related callers/last together after subject fledged" stimuli, 12 as "Related callers/last together before subject hatched" stimuli, nine as "Related callers/never lived together" stimuli, and 24 as "Unrelated callers/never lived together" stimuli. When the same individual's call was used in more than one stimulus, I used different recordings of that individual whenever possible.

To increase the chance that the subjects would be familiar with all the callers they were exposed to, I created the playback stimuli using calls from individuals that lived a maximum of 1087 m (mean = 438 ± 236 m) from the territory of the intended subject. For the treatment "Related callers/last together after subject fledged" I calculated *Caller-Subject Distance* at the time when the two callers last lived in the same group, as the purpose of this treatment was to test whether the subjects could remember having observed the callers living together. For all other treatments I calculated *Caller-Subject Distance* at the time of the experiment. Given that acorn woodpeckers regularly engage in extraterritorial forays up to at least several kilometers away from their home territory (Koenig et al. 1996), it is likely that the subjects had ample opportunity to become familiar with the callers I used as playback stimuli. *Caller-Subject Distance* did not differ significantly among any of the treatment categories (ANOVA, $F_{4,118}$=0.80, P=0.53).

Due to the difficulty of obtaining suitable recordings from specific individuals, in some cases I constructed playback stimuli using calls recorded from individuals that had died or disappeared from the study area by the time of the experiment. "Related callers/currently living together" had no stimuli with dead callers by definition, and among the remaining four treatments, "Related callers/last together after subject fledged" had more dead callers than any of the other treatments (Pearson's Chi-square test, χ^2=8.7, df=3, P=0.03).

For all treatments except "Unrelated callers/never lived together", the pair of callers in a given stimulus were genetically related. I quantified the degree of genetic relatedness using a pedigree, which was constructed from parentage assignments based on microsatellite markers (J. Haydock, unpublished data). Mean relatedness did not differ significantly across the four applicable treatment categories (ANOVA, $F_{3,115}$=0.17, P=0.92).

(d) *Construction of playback stimuli*

Playback stimuli were created in Audacity® 2.1.1. Each stimulus consisted of one min of background noise with a 10 sec fade-in, followed by two overlapping *waka* calls, followed by 30 sec of background noise, followed by the same two overlapping calls, followed by 10 sec of background noise with a fade-out applied to all 10 sec. Before being overlapped, both calls were low-pass filtered (200 Hz cutoff, 6 dB roll off or 12 dB in cases of unusually loud low-frequency noise) and normalized to -3 dB.

Waka calls consist of a series of alternating "*wa*" and "*ka*" notes, with the "*wa*" notes being much louder and longer and having clearer harmonics than the "*ka*" notes. The first and last few notes of a *waka* call are often softer and shorter and have a less distinct harmonic structure than the "*wa*" notes throughout the rest of the call. I overlapped the calls in each playback stimulus such that the first clear "*wa*" note of the second call started 0.5-1 sec after the

first clear "*wa*" note of the first call, which mimics natural *waka* choruses. I also ensured that at least one clear "*wa*" note of each call was in between the notes of the other call with no overlap, to make it easier for the subjects to hear and identify both callers. The mean duration of the pair of overlapping calls in each stimulus did not differ significantly among the five treatment categories (ANOVA, $F_{4,118}$=0.29, P=0.88).

Whenever possible, I used only recordings that were free of any overlap with other callers or other undesirable sounds. However, in some cases I had no choice but to use calls that had minor overlap with other sounds. When the other sounds were interleaved with the notes of the primary caller such that there was no actual overlap, I pasted over the undesirable sounds with background noise. When the undesirable sounds overlapped with some notes at either end of the *waka*, I deleted the affected notes. The number of notes per call is highly variable within an individual, and most of the deletions only affected a few indistinct notes at the end of the call, so altered calls were still within the natural range of variation seen within an individual. In the few cases where a deletion affected inter-note intervals or truncated the call in such a way that it appeared unnatural, I replaced the deleted notes with notes of similar duration and amplitude taken from the same call. 57.7% of playback stimuli were unmodified, 26.8% had a few indistinct soft notes at the end of the call deleted, and 15.4% had at least one full-volume note deleted and/or had deleted notes replaced with similar notes from elsewhere in the call. These manipulations are unlikely to have affected the subject's ability to recognize the callers, as individual identity is independently encoded in the harmonic structure of each "*wa*" note within a *waka* call (Yao 2008).

(e) *Measuring response to playback*

I videotaped the subject during each trial and measured the following six response variables within a three-minute period beginning with the start of the playback: latency to the first "directional" flight (flying up to higher vantage point or toward speaker), latency to the first "reaction" (flying to higher vantage point, flying toward speaker, or vocalizing), latency to the first approach to the speaker, latency to the closest approach to the speaker, distance of the first approach to the speaker, and distance of the closest approach to the speaker. I used only *Latency to Directional Flight*, *Latency to React*, and *Distance of First Approach* in the analysis, as the other response variables were highly correlated (Pearson's $r > 0.80$) with at least one of these variables.

(f) *Statistical analyses*

All analyses were conducted in R 3.5.1 (R Core Team 2018), and the significance level was set to 0.05 for all tests. I used mixed-effects Cox regression in the package "*coxme*" (Therneau 2018) to analyze *Latency to Directional Flight* and *Latency to React*, and a linear mixed model in the package "*lme4*" (Bates et al. 2015) to analyze *Distance of First Approach*. Each model contained *Treatment*, *Sex*, *Treatment*Sex*, as fixed effects and *Individual ID* as a random effect. I compared all treatments to the control (unrelated callers that never lived together) separately for males and females in the package "*emmeans*" (Lenth 2018) using Dunnett's method to adjust for multiple comparisons.

Results

(a) *Latency to Directional Flight*

Overall, the latency to the subject's first flight toward the speaker or to a higher vantage point differed among treatments (Cox regression, *Treatment*, χ^2=15.7, df=4, P=0.003). Although

the interaction term of *Treatment* and *Sex* was just short of significance (Cox regression, *Treatment*Sex*, χ^2=8.5, df=4, *P*=0.08), males showed no difference in response between unrelated callers that never lived together and any of the other treatments (Dunnett's test, all *P*>0.70) while females responded more quickly to unrelated callers that never lived together than to related callers that lived together at the time of the experiment (Dunnett's test, *P*=0.004) and to related callers that last lived together before the subject hatched (Dunnett's test, *P*=0.03). Females showed no difference in response between unrelated and related callers that never lived together (Dunnett's test, *P*=0.92), or between unrelated callers that never lived together and related callers that last lived together after the subject fledged but ≥1 year before the experiment (Dunnett's test, *P*=0.99). (Figure 3.2).

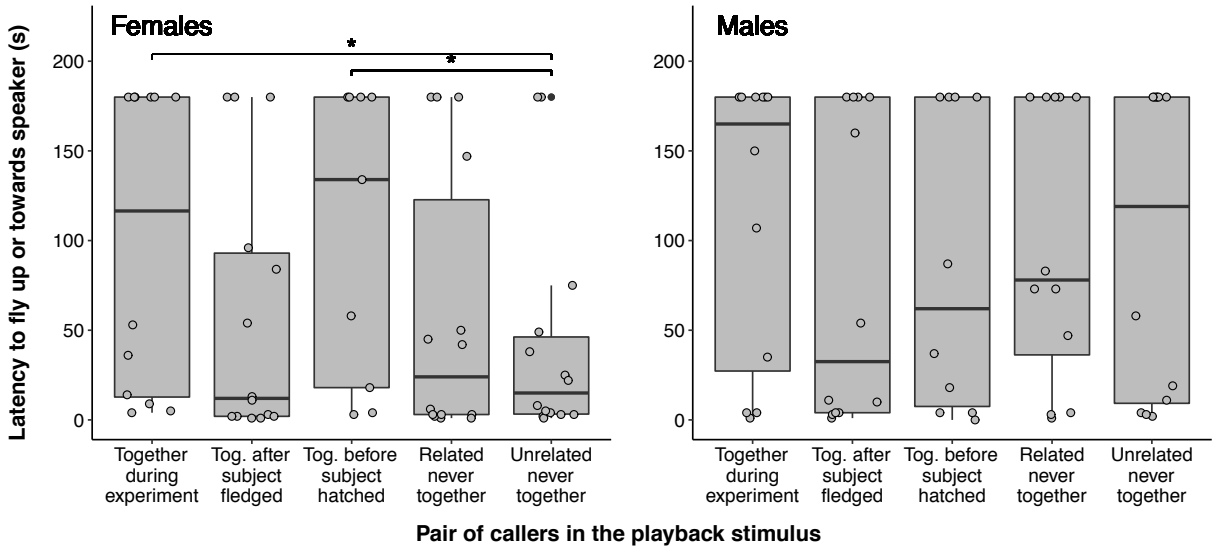


Figure 3.2. *Latency to Directional Flight* (fly up to higher vantage point or toward speaker) as a function of *Treatment*, with females and males presented separately. * indicates *P*<0.05.

(b) *Latency to React*

Males and females differed in how *Treatment* affected the latency to their first "reaction" (vocalization, flight towards the speaker, or flight to higher vantage point) (Cox regression, *Treatment*Sex*, χ^2=10.8, df=4, *P*=0.03). Females reacted more quickly to a pair of unrelated callers that never lived together than to a pair of related callers that lived together at the time of the experiment (Dunnett's test, *P*=0.04), but showed no difference in response between a pair ofunrelated callers that never lived together and the remaining treatments (Dunnett's test, all *P*≥0.14). Males showed no difference in response between unrelated callers that never lived together and any of the other treatments (Dunnett's test, all *P*≥0.46) (Figure 3.3).

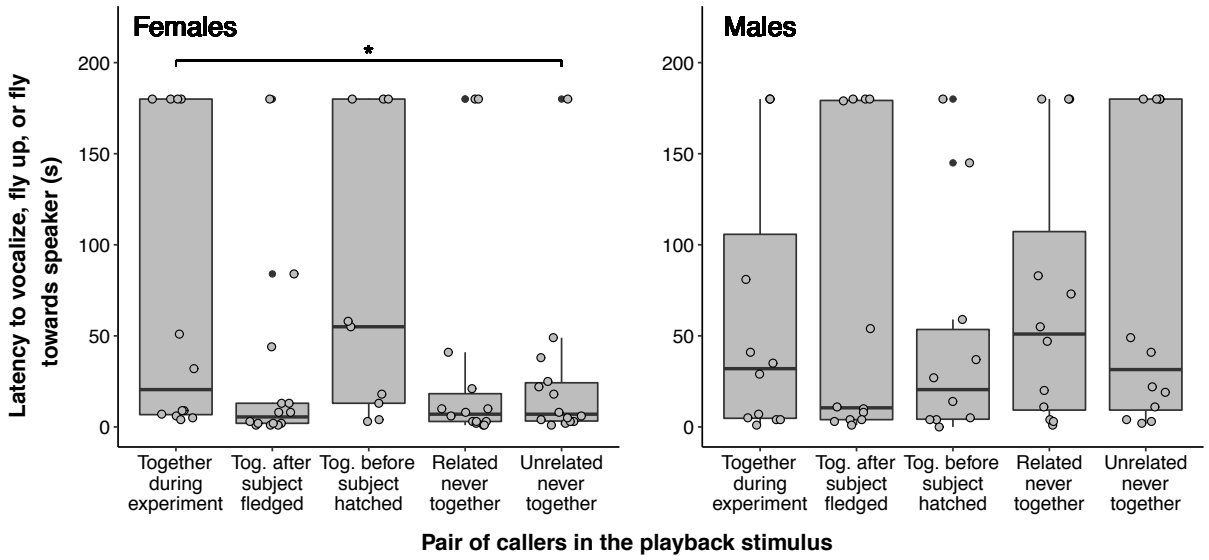


Figure 3.3. *Latency to React* (vocalize, fly up to higher vantage point, or fly toward speaker) as a function of *Treatment*, with females and males presented separately. * indicates *P*<0.05.

(c) *Distance of First Approach*

Males and females differed in how *Treatment* affected the distance of their first approach to the speaker (Cox regression, *Treatment*Sex*, χ^2=9.7, df=4, *P*=0.04). Females tended to

approach the speaker more closely in response to unrelated callers that never lived together than in response to related callers that last lived together before the subject hatched, although this was not statistically significant (Dunnett's test, P=0.054), and they showed no difference in response between unrelated callers that never lived together and the remaining treatments (Dunnett's test, all $P \geq 0.19$). Males showed no difference in response between unrelated callers that never lived together and any of the other treatments (Dunnett's test, all $P \geq 0.25$) (Figure 3.4) (Table 3.1).

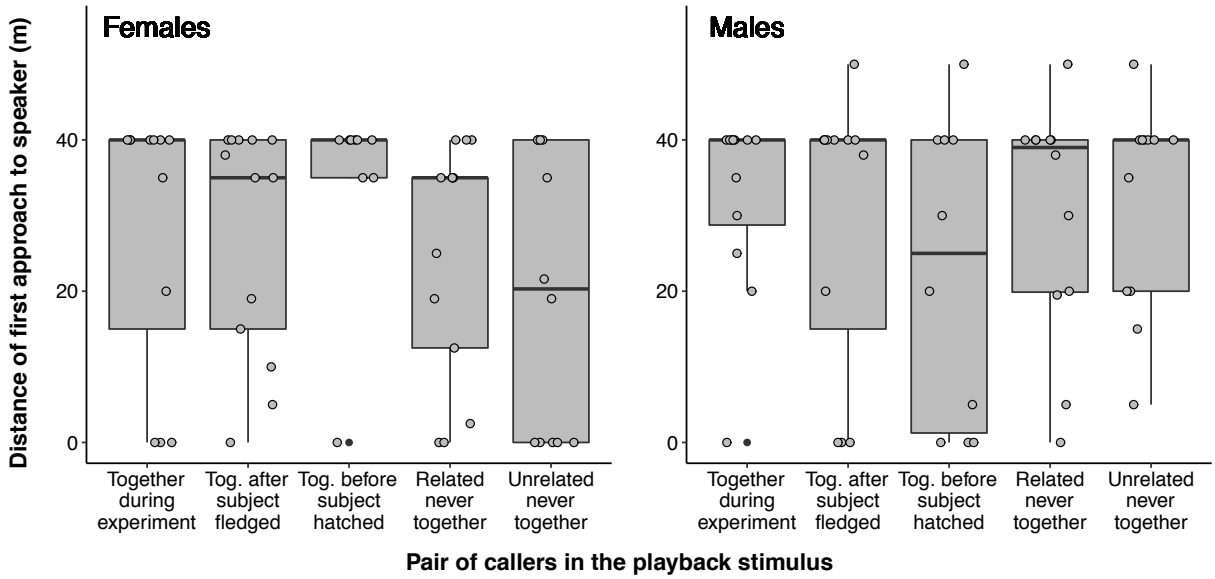


Figure 3.4. *Distance of First Approach* as a function of *Treatment*, with females and males presented separately. Shorter distances indicate a closer approach. Among females, the difference between callers that last lived together before the subject hatched and unrelated callers that never lived together was just short of significance (P=0.054).

Table 3.1. Output [Chi-square statistic (*P*-value) for fixed effects, variance for *Individual ID*] for each of the models. *Latency to Directional Flight* is defined as the latency to the subject's first flight up to a higher vantage point or towards the speaker, *Latency to React* is defined as the latency to the subject's first directional flight or vocalization, and *Distance of First Approach* is defined as the closest distance between the subject and the speaker during the subject's first approach.

Model Type	**Response**	***Treatment* (df=4)**	***Sex* (df=1)**	***Treatment*Sex* (df=4)**	***Individual ID***
Cox proportional hazards	*Latency to Directional Flight*	15.7 (0.004)	2.3 (0.13)	8.5 (0.08)	2.49
Cox proportional hazards	*Latency to React*	12.9 (0.01)	1.6 (0.21)	10.8 (0.03)	1.76
Linear	*Distance of First Approach*	7.8 (0.10)	4.0 (0.046)	9.7 (0.04)	103.7

(d) *Post-hoc analysis*

I expected that if subjects recognized the relationship between a pair of callers that last lived together before the subject hatched, they would also recognize the relationship between a pair of callers that last lived together after the subject fledged. However, while females exhibited a reduced response (indicating recognition of the relationship) to a pair of callers that last lived together before the subject hatched, their reaction to a pair of callers that last lived together after the subject fledged was just as strong as their reaction to a pair of unrelated callers that never lived together. Due to logistical constraints, playback stimuli for "Related callers/last together after subject fledged" were more likely than any other treatment to contain the call of an individual that had died or disappeared before the experiment (Pearson's Chi-square test, χ^2=8.7, df=3, *P*=0.03). To determine if this could explain the unexpectedly strong response to playbacks of callers that last lived together after the subject fledged, I ran a model for each response

variable with *Caller Alive or Dead*, *Sex*, and their interaction as a fixed effects and *Individual ID* as a random effect. *Treatment* could not be included in this model because one of the treatments (Related callers/currently living together) exclusively contained living callers, and for another treatment (Related callers/last together after subject hatched) 13/14 stimuli for female subjects contained the call of a presumed dead individual.

Females, but not males, exhibited a shorter *Latency to Directional Flight* in response to presumed dead callers than to live callers (Cox regression with Tukey's test, Females: P=0.01; Males: P=0.90) (Figure 3.5a). Similarly, females, but not males, exhibited a shorter *Latency to React* in response to presumed dead callers (Cox regression with Tukey's test, Females: P=0.01; Males: P=0.67) (Figure 3.5b). Subjects showed no difference in the distance of their first approach to the speaker in response to living vs. presumed dead callers (Cox regression: *Callers Alive or Dead*, χ^2=1.8, df=1, P=0.18; *Callers Alive or Dead*Sex*, χ^2=0.73, df=1, P=0.39).

Figure 3.5. Subjects' response to stimuli with at least one presumed dead caller vs. stimuli in which both callers were confirmed to be alive. Subjects were only exposed to callers of their own sex, so sex refers to both the sex of the subject and the sex of the caller. A: latency to the subject's first flight up to a higher vantage point or towards the speaker (*Latency to Directional Flight*). B: latency to the subject's first vocalization or first flight up to a higher vantage point or towards the speaker, whichever came first (*Latency to React*). * indicates $P<0.05$.

Discussion

This experiment suggests that female acorn woodpeckers can discriminate between a pair of callers that last lived together before the subject hatched and a pair of callers that never lived together. It also supports earlier findings from Chapter 2 that female acorn woodpeckers can discriminate between a pair of callers that currently live together in the same group and a pair of callers that have never lived together (Pardo et al. 2018). In the current chapter females were exclusively presented with female callers, whereas in Chapter 2 I presented females with a mixed-sex pair of callers, suggesting that female acorn woodpeckers are able to recognize associations between both female-male and female-female dyads from other groups (Pardo et al. 2018). In contrast, I failed to find any evidence that male woodpeckers can discriminate associations among members of other groups.

It is unclear if the failure to find differences among treatments in males was due to a genuine difference in responsiveness between male and female acorn woodpeckers, due to the fact that females received playbacks of female callers whereas males received playbacks of male callers, or simply due to a lack of statistical power. However, in other species, males and females often exhibit cognitive differences when sex differences in ecology exert differential selection pressures on cognitive development. For example, female brown-headed cowbirds (*Molothrus ater*) have more accurate spatial memory than males, because only females search for host nests in which to lay their eggs (Guigueno et al. 2014). In acorn woodpeckers, females are more likely to disperse than males, and are more likely to have to compete in a power struggle in order to secure a breeding opportunity (Hannon et al. 1985; Koenig et al. 2000). Thus, it may pay females more than males to closely monitor the interactions among individuals on other territories with whom they are competing.

Chapter 3: Recognition of relationships between individuals that no longer live together

Most studies of triadic awareness assume that animals learn the relationships between others by recognizing individual conspecifics and observing them as they interact with one another (Seyfarth and Cheney 2015). In theory, however, it might be possible to infer the relationships between third parties using simple cues of kinship or status. For example, the calls of killer whales (*Orcinus orca*) from the same matriline are more similar than the calls of whales from different matrilines, which could potentially be used to assess the degree of relatedness between third parties (Yurk et al. 2002). Similarly, paper wasps (*Polistes dominulus*) have facial markings that signal their dominance status, which could theoretically be used to assess the relative dominance ranks of third party dyads (Tibbetts and Dale 2004). If female acorn woodpeckers use kin signatures to assess the relationships among others, then inferring the existence of a relationship between individuals that no longer live in the same group would be no more cognitively demanding than inferring the existence of a relationship between individuals that currently live together. If, however, they rely on individual recognition and observation of the interactions among others to infer the existence of third party relationships, then recognizing relationships between individuals that no longer live together could be substantially more cognitively challenging then recognizing relationships between individuals that currently live together, because there is less opportunity to reinforce knowledge about relationships between individuals that do not live together. Recognizing such cross-group relationships between other individuals without the help of kin signatures would either require remembering for years that the birds involved used to live together, or it would require observing the birds interact with one another post-dispersal, which is presumably a much rarer occurrence than interactions among members of the same group.

The finding that female acorn woodpeckers did not discriminate between unrelated and genetically related callers that never lived together refutes the kin signature hypothesis. This was expected, given the previous finding that female acorn woodpeckers were able to recognize the association between male and female breeders that live in the same group but are unrelated (Pardo et al. 2018), but it provides a second basis upon which to rule out this cognitively simpler explanation. It is also unlikely that subjects used learned vocal signatures of group identity to recognize the relationships between individuals that no longer live together, because 6/9 female playback stimuli in which the callers last lived together before the subject hatched were recorded after the callers were already living in separate groups, and therefore would have been unlikely to share their prior group's learned call, even if learned group signatures exist in acorn woodpeckers.

Females discriminated between unrelated callers that never lived together and callers that used to live together but dispersed to separate groups before the subject hatched. This refutes the hypothesis that acorn woodpeckers can only recognize relationships between individuals that no longer live together if the subject had the chance to observe the callers living together in the past. Rather, it is consistent with the hypothesis that acorn woodpeckers recognize relationships between callers that no longer live together by observing the callers interact after dispersal to separate groups. Contrary to the prediction of the latter hypothesis, however, females showed no difference in response between unrelated callers that never lived together and related callers that last lived together after the subject fledged but ≥1 year before the experiment.

It is unlikely that females are of incapable of recognizing relationships between others when the individuals in question last lived together after the subject fledged, given that they are seemingly capable of recognizing such third party relationships when the individuals in question

last lived together before the subject hatched. Why then did females exhibit a reduced response (presumably indicating recognition of the relationship) to a pair of callers that last lived together before the subject hatched, but a strong response to a pair of callers that last lived together after the subject fledged? Due to logistical constraints, stimuli with callers that last lived together after the subject fledged but ≥1 year before the experiment were significantly more likely to contain the call of an individual that had died or disappeared from the study area than other stimuli, and female subjects responded more strongly to the calls of presumed dead individuals. Thus, the unexpectedly rapid response to callers that last lived together after the subject fledged but ≥1 year before the experiment can likely be explained by the fact that these callers were more likely to be dead or missing from the study area than the callers in other treatments. Together with the other results of this experiment, this provides support for the hypothesis that female acorn woodpeckers can recognize relationships between members of other groups that no longer live together, and that they do so by observing the third parties in question interact with each other after the third parties dispersed to separate groups.

Understanding the relationships between other individuals that no longer live together represents a cognitive leap beyond the forms of triadic awareness documented in previous studies, because individuals living in separate groups interact with one another much less frequently than individuals living in the same group, and therefore there is less opportunity for third parties to observe their interactions and learn about their relationship. Few studies have investigated how much input animals need to learn and retain third party knowledge. Great tits (*Parus major*) remembered the dominance relationship between two rival males for at least 15 min after witnessing a single simulated interaction between them (Peake et al. 2002), and chimpanzees (*Pan troglodytes*) remembered their own agonistic encounters for at least two hours

(Wittig et al. 2014). However, acorn woodpeckers likely do not visit their former group members every day, and even when they do visit one another, a third individual can only witness their interaction if she happens to be in the same place at the same time. Therefore, keeping track of the relationships between other individuals that live in separate groups likely requires acorn woodpeckers to retain third party knowledge for considerable lengths of time.

Social group size is often used as a proxy for social complexity, and it is often assumed that group size determines the number of third-party relationships that individuals need to monitor simultaneously (Dunbar 1992). However, this view may be biased by the fact that most research on triadic awareness has been conducted with primates, in which the primary function of triadic awareness appears to be to monitor complex competitive interactions within the social group (Seyfarth and Cheney 2015). Acorn woodpeckers live in a very different social environment, in which the most complex competitive interactions typically take place between, rather than within, social groups (Koenig 1981a; Hannon et al. 1985). In areas with sufficient suitable habitat, a radius of 438 m (the mean distance between the territories of subjects and callers in the current study) can encompass 10 acorn woodpecker groups, with a mean size of 4.76±2.58 adults each (Koenig 1981b). Therefore, although acorn woodpeckers have much smaller social groups than many primates, they likely individually recognize and monitor the relationships between at least 40-50 conspecifics, which is comparable to many primate societies (Dunbar et al. 2018). A recent finding that brain size decreases with social group size in woodpeckers, opposite of the pattern found in primates, further supports the idea that social group size *per se* is not the primary selective force on the evolution of cognition in woodpeckers (Fedorova et al. 2017). Investigating social cognition in "solitary" and pair-living woodpeckers

could have important implications for our understanding of social complexity and the evolution of intelligence.

This study provides the first empirical evidence that a wild animal can recognize relationships between conspecifics that do not currently live together. The results highlight the importance of considering how "extended" social environments beyond the core social group may have shaped the evolution of social cognition. Future work should investigate whether other species can recognize relationships between third parties that no longer live together, and explore the relative influence of intra- vs. inter-group social selection pressures on cognitive evolution.

REFERENCES

Akçay Ç, Reed VA, Campbell SE, Templeton CN, Beecher MD. 2010. Indirect reciprocity: song sparrows distrust aggressive neighbours based on eavesdropping. Anim. Behav. 80:1041–1047.

Akçay Ç, Swift RJ, Reed VA, Dickinson JL. 2013. Vocal kin recognition in kin neighborhoods of western bluebirds. Behav. Ecol. 24:898–905.

Bachmann C, Kummer H. 1980. Male assessment of female choice in hamadryas baboons. Behav. Ecol. Sociobiol. 6:315–321.

Baptista LF, Schuchmann KL. 1989. Song learning in the Anna hummingbird (*Calypte anna*). Ethology 84:15–26.

Bates D, Mächler M, Bolker BM, Walker SC. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1–48.

Boeckle M, Bugnyar T. 2012. Long-term memory for affiliates in ravens. Curr. Biol. 22:801–806.

Borgeaud C, van de Waal E, Bshary R. 2013. Third-party ranks knowledge in wild vervet monkeys (*Chlorocebus aethiops pygerythrus*). PLoS One 8:e58562.

Braun A, Bugnyar T. 2012. Social bonds and rank acquisition in raven nonbreeder aggregations. Anim. Behav. 84:1507–1515.

Bruck JN. 2013. Decades-long social memory in bottlenose dolphins. Proc. R. Soc. B 280:20131726.

Cheney DL, Seyfarth RM. 1980. Vocal recognition in free-ranging vervet monkeys. Anim. Behav. 28:362–367.

Cheney DL, Seyfarth RM. 1982. Recognition of individuals within and between groups of free-ranging vervet monkeys. Am. Zool. 22:519–529.

Cheney DL, Seyfarth RM, Silk JB. 1995. The responses of female baboons (*Papio cynocephalus ursinus*) to anomalous social interactions: evidence for causal reasoning? J. Comp. Psychol. 109:134–141.

Cook RG, Levison DG, Gillett SR, Blaisdell AP. 2005. Capacity and limits of associative memory in pigeons. Psychon. Bull. Rev. 12:350–358.

Crockford C, Wittig RM, Seyfarth RM, Cheney DL. 2007. Baboons eavesdrop to deduce mating opportunities. Anim. Behav. 73:885–890.

Cuthbert JL, Mennill DJ. 2007. The duetting behavior of Pacific Coast plain wrens. Condor 109:686–692.

Dickinson JL, Koenig WD, Pitelka FA. 1996. Fitness consequences of helping behavior in the western bluebird. Behav. Ecol. 7:168–177.

Draganoiu TI, Ravaux L, Moreau A, Mathevon N, Bonckaert W. 2014. Song stability and neighbour recognition in a migratory songbird, the black redstart. Behaviour 151:435–453.

Dunbar RIM. 1992. Neocortex size as a constraint on group size in primates. J. Hum. Evol. 22:469–493.

Dunbar RIM, Mac Carron P, Shultz S. 2018. Primate social group sizes exhibit a regular scaling pattern with natural attractors. Biol. Lett. 14:20170490.

Dunbar RIM, Shultz S. 2007. Evolution in the social brain. Science. 317:1344–1347.

Elie JE, Mariette MM, Soula HA, Griffith SC, Mathevon N, Vignal C. 2010. Vocal communication at the nest between mates in wild zebra finches: a private vocal duet? Anim. Behav. 80:597–605.

Emery NJ, Seed AM, von Bayern AMP, Clayton NS. 2007. Cognitive adaptations of social bonding in birds. Philos. Trans. R. Soc. B Biol. Sci. 362:489–505.

Engh AL, Siebert ER, Greenberg DA, Holekamp KE. 2005. Patterns of alliance formation and postconflict aggression indicate spotted hyaenas recognize third-party relationships. Anim. Behav. 69:209–217.

Fedorova N, Evans CL, Byrne RW. 2017. Living in stable social groups is associated with reduced brain size in woodpeckers (*Picidae*). Biol. Lett. 13:20170008.

Godard R. 1991. Long-term memory of individual neighbours in a migratory songbird. Nature 350:228–229.

Gonçalves A, Biro D. 2018. Comparative thanatology, an integrative approach: exploring sensory/cognitive aspects of death recognition in vertebrates and invertebrates. Philos. Trans. R. Soc. B 373:20170263.

Grosenick L, Clement TS, Fernald RD. 2007. Fish can infer social rank by observation alone. Nature 445:429–432.

Guigueno MF, Snow DA, Macdougall-Shackleton SA, Sherry DF. 2014. Female cowbirds have more accurate spatial memory than males. Biol. Lett. 10:20140026.

Hanazuka Y, Shimahara N, Tokuda Y, Midorikawa A. 2013. Orangutans (*Pongo pygmaeus*) remember old acquaintances. PLoS One 8:e82073.

Hannon SJ, Mumme RL, Koenig WD, Pitelka FA. 1985. Replacement of breeders and within-group conflict in the cooperatively breeding acorn woodpecker. Behav. Ecol. Sociobiol. 17:303–312.

Hatchwell BJ, Ross DJ, Fowlie MK, McGowan A. 2001. Kin discrimination in cooperatively breeding long-tailed tits. Proc. R. Soc. B 268:885–890.

Haydock J, Koenig WD, Stanback MT. 2001. Shared parentage and incest avoidance in the cooperatively breeding acorn woodpecker. Mol. Ecol. 10:1515–1525.

Humphrey NK. 1976. The social function of intellect. In: Bateson PPG, Hinde RA, editors. Growing Points in Ethology. Cambridge: Cambridge University Press. p. 303–317.

Insley SJ. 2000. Long-term vocal recognition in the northern fur seal. Nature 406:404–405.

Jungwirth A, Walker J, Taborsky M. 2015. Prospecting precedes dispersal and increases survival chances in cooperatively breeding cichlids. Anim. Behav. 106:107–114.

Keenan S, Mathevon N, Stevens JMG, Guéry JP, Zuberbühler K, Levréro F. 2016. Enduring voice recognition in bonobos. Sci. Rep. 6:22046.

Kendrick KM, da Costa AP, Leigh AE, Hinton MR, Peirce JW. 2001. Sheep don't forget a face. Nature 414:165–166.

Koenig WD. 1981a. Space competition in the acorn woodpecker: power struggles in a cooperative breeder. Anim. Behav. 29:396–409.

Koenig WD. 1981b. Reproductive success, group size, and the evolution of cooperative breeding in the acorn woodpecker. Am. Nat. 117:421–443.

Koenig WD, Haydock J, Stanback MT. 1998. Reproductive roles in the cooperatively breeding acorn woodpecker: incest avoidance versus reproductive competition. Am. Nat. 151:243–255.

Koenig WD, Hooge PN, Stanback MT, Haydock J. 2000. Natal dispersal in the cooperatively breeding acorn woodpecker. Condor 102:492–502.

Koenig WD, Mumme RL. 1987. Population Ecology of the Cooperatively Breeding Acorn Woodpecker. Princeton, NJ: Princeton University Press.

Koenig WD, Van Vuren D, Hooge PN. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. Trends Ecol. Evol. 11:514–517.

Koenig WD, Walters EL. 2016. Provisioning patterns in the cooperatively breeding acorn woodpecker: does feeding behaviour serve as a signal? Anim. Behav. 119:125–134.

Koenig WD, Walters EL, Haydock J. 2011. Variable helper effects, ecological conditions, and the evolution of cooperative breeding in the acorn woodpecker. Am. Nat. 178:145–158.

Koenig WD, Walters EL, Haydock J. 2016. Acorn woodpeckers: helping at the nest, polygynandry, and dependence on a variable acorn crop. In: Koenig WD, Dickinson JL, editors. Cooperative Breeding: Studies of Ecology, Evolution, and Behavior. Cambridge, UK: Cambridge University Press. p. 217–236.

Kubenova B, Konecna M, Majolo B. 2017. Triadic awareness predicts partner choice in male–infant–male interactions in Barbary macaques. Anim. Cogn. 20:221–232.

Lazaro-Perea C. 2001. Intergroup interactions in wild common marmosets, *Callithrix jacchus*: territorial defence and assessment of neighbours. Anim. Behav. 62:11–21.

Lenth R. 2018. emmeans: estimated marginal means, aka least-squares means. R package version 1.3.5. <https://CRAN.R-package.org/package=emmeans>.

MacRoberts MH, MacRoberts BR. 1976. Social organization and behavior of the acorn woodpecker in central coastal California. Ornithol. Monogr. 21:1–115.

Massen JJM, Pašukonis A, Schmidt J, Bugnyar T. 2014. Ravens notice dominance reversals among conspecifics within and outside their social group. Nat. Commun. 5:3679.

Mateo JM, Johnston RE. 2000. Retention of social recognition after hibernation in Belding's ground squirrels. Anim. Behav. 59:491–499.

Matthews S, Snowdon CT. 2011. Long-term memory for calls of relatives in cotton-top tamarins (*Saguinus oedipus*). J. Comp. Psychol. 125:366–369.

Maynard DF, Ward K-AA, Doucet SM, Mennill DJ. 2012. Calling in an acoustically competitive environment: duetting male long-tailed manakins avoid overlapping neighbours but not playback-simulated rivals. Anim. Behav. 84:563–573.

McComb K, Moss C, Sayialel S, Baker L. 2000. Unusually extensive networks of vocal recognition in African elephants. Anim. Behav. 59:1103–1109.

Moss CJ, Poole JH. 1983. Relationships and social structure in African elephants. In: Hinde RA, editor. Primate social relationships: an integrated approach. Oxford, UK: Blackwell Science, Inc. p. 315–325.

Nottebohm F. 1972. The origins of vocal learning. Am. Nat. 106:116–140.

Palombit RA, Seyfarth RM, Cheney DL. 1997. The adaptive value of "friendships" to female baboons: experimental and observational evidence. Anim. Behav. 54:599–614.

Pardo MA, Sparks EA, Kuray TS, Hagemeyer ND, Walters EL, Koenig WD. 2018. Wild acorn woodpeckers recognize associations between individuals in other groups. Proc. R. Soc. B 285:20181017.

Paz-y-Miño C G, Bond AB, Kamil AC, Balda RP. 2004. Pinyon jays use transitive inference to predict social dominance. Nature 430:778–781.

Peake TM, Terry AMR, Mcgregor PK, Dabelsteen T. 2002. Do great tits assess rivals by combining direct experience with information gathered by eavesdropping? Proc. R. Soc. B Biol. Sci. 269:1925–1929.

Perry S, Barrett HC, Manson JH. 2004. White-faced capuchin monkeys show triadic awareness in their choice of allies. Anim. Behav. 67:165–170.

Pitcher BJ, Harcourt RG, Charrier I. 2010. The memory remains: long-term vocal recognition in Australian sea lions. Anim. Cogn. 13:771–776.

Price JJ. 1999. Recognition of family-specific calls in stripe-backed wrens. Anim. Behav. 57:483–492.

Proops L, Mccomb K, Reby D. 2008. Cross-modal individual recognition in domestic horses (*Equus caballus*). Proc. Natl. Acad. Sci. 106:947–951.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org>.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org>.

Radford AN. 2003. Territorial vocal rallying in the green woodhoopoe: influence of rival group size and composition. Anim. Behav. 66:1035–1044.

Seyfarth RM, Cheney DL. 2015. Social cognition. Anim. Behav. 103:191–202.

Stoddard PK. 1996. Vocal recognition of neighbors by territorial passerines. In: D. E. Kroodsma, E. H. Miller, editors. Ecology and Evolution of Acoustic Communication in Birds. Ithaca, NY: Cornell University Press. p. 356–374.

Therneau T. 2015. A package for survival analysis in S. R package version 2.38. <https://CRAN.R-project.org/package=survival>.

Therneau TM. 2018. coxme: Mixed effects cox models. R package version 2.2-10. <https://CRAN.R-project.org/package=coxme>.

Tibbetts EA, Dale J. 2004. A socially enforced signal of quality in a paper wasp. Nature 432:218–222.

Toth CA, Mennill DJ, Ratcliffe LM. 2012. Evidence for multicontest eavesdropping in chickadees. Behav. Ecol. 23:836–842.

Wittemyer G, Douglas-Hamilton I, Getz WM. 2005. The socioecology of elephants: analysis of the processes creating multitiered social structures. Anim. Behav. 69:1357–1371.

Wittig RM, Crockford C, Langergraber KE, Zuberbühler K. 2014. Triadic social interactions operate across time: a field experiment with wild chimpanzees. Proc. R. Soc. B Biol. Sci. 281:20133155.

Wittig RM, Crockford C, Wikberg E, Seyfarth RM, Cheney DL. 2007. Kin-mediated reconciliation substitutes for direct reconciliation in female baboons. Proc. R. Soc. B Biol. Sci. 274:1109–1115.

Yao Y. 2008. Studies of vocal communications in cooperatively breeding acorn woodpeckers (*Melanerpes formicivorus*). PhD Disser., UCLA, Los Angeles, CA.

Yurk H, Barrett-Lennard LG, Ford JKB, Matkin CO. 2002. Cultural transmission within maternal lineages: vocal clans in resident killer whales in southern Alaska. Anim. Behav. 63:1103–1119.